A STUDY ON BACTERIAL AND FUNGAL ISOLATES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN PATIENTS WITH CHRONIC OSTEOMYELITIS IN A TERTIARY CARE HOSPITAL

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CERTIFICATE

This is to certify that this dissertation titled **"A STUDY ON BACTERIAL AND FUNGAL ISOLATES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN PATIENTS WITH CHRONIC OSTEOMYELITIS IN A TERTIARY CARE HOSPITAL"** is a bonafide record of work done by **DR.C.DEVI,** during the period of her Post graduate study from 2010 to 2013 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-600003, in partial fulfillment of the requirement for M.D. MICROBIOLOGY degree Examination of The Tamilnadu Dr.M.G.R. Medical University to be held in April 2013.

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DECLARATION

I declare that the dissertation entitled **"A STUDY ON BACTERIAL AND FUNGAL ISOLATES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN PATIENTS WITH CHRONIC OSTEOMYELITIS IN A TERTIARY CARE HOSPITAL"** is submitted by me for the degree of M.D. is the record work carried out by me during the period of October 2011 to September 2012 under the guidance of **Prof.Dr.S.THASNEEM BANU, M.D.** Professor of Microbiology, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D., Microbiology (Branch IV) examination to be held in April 2013.

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INTRODUCTION

The word Osteomyelitis is a combination of Greek word “Osteon” meaning bone and “Myelos” meaning marrow plus the suffix “Itis” meaning inflammation. [1]

Osteomyelitis is acquired in three ways. They are direct seeding of microorganisms into bone due to trauma or surgery,haematogenous spread of microorganisms from the focus of infection elsewhere in the body and spread from surrounding infected softtissues and joints[2] .

In t he infected bo ne, the infect io n may be unifocal or mult ifocal. I n unifocal invo lvement, only a single regio n of t he bone is affected. I n mult ifocal involvement, more t han o ne regio n o f bone such as marrow, perio steum, cortex and t he surrounding so ft tissue are involved. Commonly t he infect ion is mo no microbial. Infect ion due to mult iple organisms [3] are usually seen in pat ient s with Diabetes mellit us wit h ulcer in t he foot.

Chronic Osteo myelit is o f lo ng bo nes is o ften t he consequence o f an open, comminuted fracture and inadequately treated infect ion of t he fracture site. Rarely it occurs as a co mplicat io n of acute osteomyelit is now-a-days.

The following six components characterise chronic osteomyelitis.

1) Sequestrum formation or sclerosis.

2) Radiological changes seen in bone due to infection for 6 weeks or longer.

3) Relapse or persistence of infection after initial treatment.

4) Osteomyelitis due to foreign bodies.

5) Osteomyelitis in association with peripheral vascular disease.

6) Organisms that produce chronic disease (e.g Mycobacterium tuberculosis)

The pathological process involves the necrosis of bone, granulation tissue formation, absorption of necrotic cancellous bone ,replacement by new bone formation and cicatrix formation due to neighbouring softtissue destruction. Sequestrum is the dead bone that has been separated from the living bone. Involucrum is the new bone formed.

The commonest presenting symptoms are persistent pain and chronic intermittent discharge through sinuses. Bone debris and sequestra find exit through multiple openings in an involucrum, go through the sinus tracts and present to the surface. In children, after discharge of sequestrum, the sinus is closed and the cavity is filled with new bone. In adults, the sinus is not closed and the persistence of viable pathogens in cavities for a longer period leads to reactivation of infection at any time.

Chronic osteomyelitis still remains a major challenging problem in our country .In the majority of cases, it is due to delay, neglect or inadequate

treatment of acute hematogenous osteomyelitis and compound fractures. [4]

The usual complications of chronic osteomyelitis are reduced rate of growth,pathological fracture, septic arthritis, lengthening of bone, contracture of muscles. Other rare complications are formation of epithelioma, secondary amyloidosis [5] ,squamous cell carcinoma in scar tissue(<1%).

Chronic osteomyelitis is a disease which is difficult to eradicate completely. There may be subsidence of systemic symptoms,but the cavities containing purulent material, infected granulation tissue or sequestrum act as foci of infection. There may be recurrent acute flare-ups occuring at indefinite intervals over months and years. To achieve eradication of the disease,aggressive surgical debridement with curettage of cavities, filling of cavities with soft tissues and effective antimicrobial treatment is required [6] .

The pattern and behaviour of organisms are constantly changing under the pressure of newer antibiotics [7] .As a result the wonder drugs of fifties have been relegated to a position of limited usefulness today. With this background, it is felt worthwhile to study the spectrum of organisms causing osteomyelitis and their antimicrobial susceptibility pattern.

REVIEW OF LITERATURE

GENERAL CONCEPTS OF THE DISEASE

Historical features

Years before, the surgeons charaka and sushruta (2500 BC) made the first documentation of Osteomyelitis in their writings. Subsequently Hippocrates accurately described it as an infection of the bony sequestrum. Sequestrum formation was first described by the physician John Hunter. An Egyptian HEARST PAPYRUS introduced casts, made of starch such as barley flour that would harden, similar to today's plaster of paris. Edwin Smit h Papyrus (Paul D, 1999) was the one who initially recognised the danger of infection with open fractures. Historically Osteomyelitis was present in war scenarios,where the most frequent cause of Osteomyelitis was trauma. In the last few decades Osteomyelitis occurred after surgical interventions for the treatment of fractures.

Osteomyelitis is a condition in which there is an acute or chronic inflammatory process occuring in the bone and its structure due to infection with pyogenic organisms. It is an infection in the bonemarrow that spread to the bone cortex and periosteum via the haversian canals. [8] As the bone cortex is thin in the region of metaphysis, microorganisms get easy access to the periosteum. Exogenous or Haematogenous spread are the most common routes of infection that occurs in the bone. Exogenous Osteomyelitis is caused by penetrating wounds, Compound fractures and Simple fractures treated surgically with Open reduction and other Orthopaedic appliances like plates, nails, screws and pins which introduce microbial infection

directly into the bones.The haematogenous form is due to bacteremia.

Various classification systems for Osteomyelitis are available.They are Waldvogel and Cierny-Mader classification system. Waldvogel[9] classified the disease as haematogenous,contiguous and chronic .Cierny Mader [10] classification system is for staging Chronic Osteomyelitis. This staging system depends on the status of disease process and the conditions of the patients and their treatments. Staging is done both anatomically and physiologically. Under anatomical type there are four stages as medullary, superficial, localized and diffuse. Under physiological type, healthy hosts are classified as A host, hosts with systemic, local and both local and systemic compromising factors as Bs, Bl, Bls and hosts in whom treatment made worse than the disease as C host. Systemic factors include malnutrition, diabetes mellitus, hepatic or renal failure, immunosuppression. Local factors include venous stasis, chronic lymphedema, arteritis, small vessel disease, tobacco abuse.

1. ACUTE HAEMATOGENOUS OSTEOMYELITIS

This type of Osteomyelitis is most commonly seen in children [11,12] . Over the last several decades the incidence of acute Osteomyelitis has dramatically decreased due to higher standard of living and improved hygiene.

EPIDEMIOLOGY

Children are most commonly affected. The most common age group affected are children less than two years old and children eight to twelve years. Hence Bimodal[13] age distribution is seen. It is more common in males,

occuring three to four times more frequent than in females. Blyth etal, reported a 70% decrease in the incidence of Osteomyelitis over the past few years. A higher standard of living and improved hygiene probably have contributed to this trend.

PATHOGENESIS

It involves mostly the metaphyses of long bones. The tibia followed by femur [14] are the two most common bones affected. . The predilection of infection to the metaphyseal region is explained by its peculiar anatomy.Metaphyseal capillaries lack phagocytic cells, the hairpin bend like anatomy of the nutrient capillaries near the growth plate favours vascular stasis. A minor trauma triggers the event by producing a small haematoma which in turn cause vascular obstruction leading to necrosis of the affected part of the bone. This area will serve as a bed for the organism resulting from transient bacteremia. It is also found in the adult population. The infection involving the cortex will lift the periosteum thereby leading to the formation of soft tissue abscess. After some time the abscess will lead to sinus tract formation connecting the sequestrum to skin. Vertebral osteomyelitis is becoming more common now-a-days due to intravenous drug use,increasing life expectation and increased nosocomial infection.

CAUSATIVE ORGANISMS

The spectrum of microorganisms are variable according to the type of Osteomyelitis, epidemiology, age of the patient, comorbidity, microbiological technique and duration of infection. [17] Microbiological culture and susceptibility testing should always be performed in order to optimize the

antimicrobial therapy.

In infants less than one year, Group B streptococci,Sstaphylococcus aureus and Escherichia coli are the common organisms isolated. In children aged 1 to 16 years S.aureus, Streptococcus pyogenes and Haemophilus influenzae are the common causative organisms. In adults more than 16 years Staphylococcus epidermidis, S.aureus, Pseudomonas aeruginosa, Serratia marcescens and E.coli are the commonly isolated organisms.In acute Osteomyelitis, over 50 % of clinical specimens contains a single organism. [18]

Common organisms isolated in specific condition

Community acquired methicillin resistant Staphylococcus aureus (MRSA) is the important cause of acute osteomyelitis in children. [19] The cytotoxin which is present in community acquired MRSA is Panton valentine leucocidin (PVL).In children osteomyelitis due to PVL positive S.aureus presents with more aggressive form with multifocal involvement when compared with PVL negative S.aureus. [20] Common organisms in neonates are group B Streptococci and Escherichia coli.Candida spp and Pseudomonas aeruginosa are commonly encountered in IV drug abusers and patients with indwelling catheters. Haemophilus influenzae type b, a common cause of long bone osteomyelitis , has become rare due to the development and widespread use of effective vaccine in children. [21]

CLINICAL FEATURES

In children, the early sign is pseudoparalysis(failure to move the affected extremity) associated with fever and pain during passive movement

of the affected limb.The signs of inflammation normally disappear within 5-7 days. Point tenderness is present if periosteum gets involved.

Neonates, when compared to other ages usually presents with non-specific symptoms, resulting in delayed recognition and leading to serious musculoskeletal sequelae [22]

DIAGNOSIS

Early diagnosis plays an important role in the management of Osteomyelitis. In children, the diagnosis is made with compatible radiologic and clinical findings with positive blood cultures. In adults, a CT-guided aspirate or open biopsy is often necessary to establish a definitive diagnosis.

RADIOGRAPHIC FINDINGS

It is well known that the typical lytic and periosteal bone changes of acute Osteomyelitis do not appear for 10 to 12 days following the onset of illness [23]

TREATMENT

Appropriate antibiotics for adequate duration is the mainstay of treatment for acute osteomyelitis. The principles of treatment proposed by Nade are [24]

1) An appropriate antibiotic should be administered before the formation of pus.

2) Antibiotics cannot penetrate avascular tissues and abscesses ,so these areas require surgical debridement.

3) Surgical treatment should not damage already

avascular bone and soft tissue.

4) Antibiotics should be continued even after surgical treatment.

Children are initially given parenteral therapy for 3-10 days .Then appropriate oral therapy is chosen to provide high level of antibiotic concentration which will help to eradicate the offending organism[25] .Oral therapy with Cloxacillin and Clindamycin were as effective as parenteral penicillin in another study[26] .

COMPLICATIONS

In the preantibiotic era, 50 % mortality was seen in acute osteomyelitis due to sepsis with metastatic abscesses. Today this is not the scenario.Acute hematogenous S.aureus Osteomyelitis in children can lead to pathologic fractures. This can occur in about 5 % of cases with a 72 day mean time from disease onset to fracture. Other complications include Bone abscess, Bacteremia, Septic arthritis, Chronic infection. [27] The percentage of children developing chronic infection as a complication is 3-5%.

2. CHRONIC OSTEOMYELITIS

In Chronic Osteomyelitis, the common signs and symptoms are bone loss and persistent drainage from sinus tracts. Cavities in the bone act as the nidus for a persistent infection leading to a chronic condition. When the original acute bone infection has subsided, it may persist as a low grade infection subjected to repeated recrudescences of the acute process over many months or years. The majority of cases of Chronic Osteomyelitis were as a result of post traumatic infections due to compound fracture, crush injury,

surgical procedures and diabetic infections of feet [28] .

EPIDEMIOLOGY

In the preantibiotic era, Osteomyelitis is associated with high mortality ,which has come down after the advent of antibiotics. The incidence of Chronic Osteomyelitis after compound fracture varies between 2% to 16%. This depends upon the grade of injury and the type of treatment given. Now-a-days Chronic Osteomyelitis are due to trauma and surgery rather than due to haematogenous spread.Osteomyelitis may be localized or may involve the periosteum,cortex,marrow of the bone. The lower limb bones are commonly involved. Nosocomial osteomyelitis is no longer rare.

PATHOGENESIS

Role of Microbes

The initial event which makes the infection to get localized is adhesion. Staphylococcus aureus strains possess receptors for collagen, fibrinogen, fibronectin, bone sialoprotein, and heparin sulphate [29] . Trauma or injury will expose the binding sites for the organism. For Staphylococcus aureus, the polysaccharide pseudocapsule forms strong links between the bacterial cell and bone. The synthesis of capsule leads to the formation of biofilm. In the biofilm, microcolonies are formed by bacteria that are connected to each other and the surrounding environment. The glycocalyx acts to protect the organism from host defense mechanisms and also from the antibiotics[30] . Glycocalyx interferes with phagocytosis by connecting the teichoic acid moiety which enhances opsonization and acts by consuming or covering and altering the configuration of complement. Staphylococcus

aureus cell wall contains protein A which functions as a virulence factor by interfering with opsonization and ingestion of organisms by polymorphonuclear cells, activating complement and eliciting immediate and delayed hypersensitivity reactions.

Pathology

Sequestrum development is followed by reactive new bone (the involucrum) formation by the periosteum around the sequestrum[31].

In diabetics, if bone can be seen or probed through an ulcer there is higher probability of underlying osteomyelitis. Bone biopsies from infections that have spread to a bone from a contiguous focus or that are associated with poor circulation especially in patients with diabetes are likely to yield multiple isolates. [32]

CLINICAL FEATURES

The patient presents with pain, pyrexia, redness and tenderness which have recurred or with a discharging sinus. Tissues are thickened and puckered or folded in, where a scar or sinus is attached to the underlying bone [33]. In post traumatic Osteomyelitis the bone may be deformed or ununited.

BACTERIOLOGY OF CHRONIC OSTEOMYELITIS

S.H.Sheely et al[34] states **Staphylococcus aureus** was most commonly isolated followed by gram negative bacilli, Coagulase negative Staphylococcus and Anaerobes. Other organisms are Pseudomonas, Escherichia coli, Klebsiella and Proteus. Of all the gram negative organisms, **Klebsiella** causes the most extensive bone destruction[31].

Proteus species can produce progressive, unrelenting, destructive lesions of bone. Four types are involved. Proteus mirabilis is the most common type. Salmonella osteomyelitis, a complication of typhoid fever is a well recognized but uncommon clinical entity. It occurs in association with sickle cell disease or other disorders of hemoglobin. It is characterized by multiple bone involvement [35] . In patients with hematogenous osteomyelitis the incidence of salmonella osteomyelitis is less than 1%. Salmonella typhi and Salmonella paratyhpi B are the two strains most frequently implicated [36] .

Pseudomonas causing bacteremia either by the infection of contaminated illicited drugs or as a result of infective endocarditis in the drug addict population leads to vertebral osteomyelitis [37] . Vertebral osteomyelitis caused by pseudomonas aeruginosa has also been reported in elderly persons suffering from urinary tract infections [38] . Pseudomonas aeruginosa may also involve the pubic symphysis in addicts. Brucella causes bone infection in 10% of patients with brucellosis. The vertebrae are the common site involved.

A rare bacteria, Arcanobacterium hemolyticum[39] , in an apparent case of tubercular osteomyelitis has been revealed. BCG vaccination osteomyelitis [40] seems to occur in persons with apparently normal immunity.

In Anaerobic Osteomyelitis- anaerobic cocci were isolated more frequently, and the most common of these was peptostreptococci. Bacteroides species were the most frequently grown gram negative anaerobic organism[35] with Bacteroides fragilis being the most common.

DIAGNOSIS

Due to lack of signs and symptoms, there is difficulty in diagnosing the Chronic Osteomyelitis [41] . Reduced blood supply to the bone leads to slow death of bone,which may lasts for years. Hence for diagnosing this disease,a multidisciplinary approach is required, clinical examination, laboratory tests and imaging studies. Due to chronic inflammation there is elevation of Erythrocyte sedimentation rate.The leucocyte count is generally normal. The C-reactive protein is elevated and non specific.Histopathological and microbiological examination of the infected bone by obtaining a **bone biopsy** specimen is considered as the **gold standard** for diagnosing this disease [42] The material from drainage of abscess, discharging sinuses, curettage of cavities and sequestrum should be obtained using swabs and processed for identification of bacteria.

COLLECTION AND TRANSPORT OF SPECIMEN

The site of specimen collection should be thoroughly cleaned with normal saline and then material is obtained from the depth of the sinus. The swabs should be transported to the laboratory without delay. The swabs should be transported in thioglycollate broth for anaerobic culture.

PROCESSING OF SPECIMENS

Microscopic examination

Smears should be made from the swab. Gram staining should be done and examined under the microscope for the presence of epithelial cells, pus cells, RBCs, bacteria and yeast cells. After inoculating on routine plating media the samples should be inoculated in Lowenstein Jensen medium to identify Mycobacterium tuberculosis. Specimens should be processed for

anaerobic culture also.

Culture

The specimen should be inoculated on

1) MacConkey agar plate

2) Blood agar plate

3) Chocolate agar plate

The plates should be incubated at 37°C aerobically for 24 hours. If growth is observed, colony morphology and gram stain morphology are studied.

The gram stain morphology showed gram positive cocci in clusters, the following tests are done. Catalase test, coagulase test - slide & tube, ureàse test and mannitol fermentation (aerobic and anerobic). Other special tests are also done to confirm the organisms.

If gram negative bacilli are seen the colonies are subjected to the following tests; catalase, oxidase, Hanging drop test for motility, citrate, urease, triple sugar iron and sugar fermentation tests. Other special tests are also done to confirm the organisms. The identification is done upto species level. Antibiotic sensitivity should be performed for all isolates by Kirby Bauer's disc diffusion technique on Mueller Hinton agar. Sensitivity and resistance pattern of the organisms are studied.

The use of 18-F fluorodeoxyglucose positron emission tomography

imaging showed a high degree of accuracy in diagnosing osteomyelitis. MRI is more sensitive than CT in diagnosis. In certain circumstances, these imaging modalities lack specificity. Due to their high sensitivities, they are mostly used to rule out osteomyelitis.

In a non diabetic patient, bone biopsy for finding the causative organism and its antimicrobial sensitivity pattern is taken if there are radiographic changes in bone suggestive of osteomyelitis. Radiographic changes may not revert even after the patient has started receiving appropriate antibiotic therapy. Three- phase bone or to indium- labeled white cell scan is done if the radiograph is normal and still there is suspicion of osteomyelitis. In general CT scan and MRI are less frequently used to diagnose osteomyelitis, but these are often used to determine the extent of infection and whether these are collections of pus that are amenable to drainage.The 'gold standard' specimen for diagnosing osteomyelitis is bone biopsy specimen. Sinus tract cultures are not reliable for pedicting gram negative organisms causing osteomyelitis [43] . In most cases antibiotic treatment is based on culture from deep bone biopsies or debrided tissues during surgery and their antibiotic susceptibilities. An earlier diagnosis of osteomyelitis may be achieved with **radio nucleotide** scanning.

MANAGEMENT OF CHRONIC OSTEOMYELITIS

While antimicrobial therapy is desirable in the control of Osteomyelitis, surgery remains the therapeutic and diagnostic procedure [6] .

Nelson et al states, administration of appropriate antibiotics for a

minimum of 3 weeks provides an excellent response and there is no progression to chronic condition.

Antimicrobial Therapy

Staphylococcus aureus is the common organism isolated. **Vancomycin** is the drug of choice for the strains that are resistant to both ampicillin and methicillin. Recently , **Linezolid** has evolved as a better drug against **MRSA** because of its increased oral bioavailability and good bone penetration.Prolonged use of linezolid has been associated with significant pancytopenia, peripheral neuropathy, optic neuritis[44,45] . **Daptomycin,** recently approved drug has bactericidal activity. Its utility in the treatment of vancomycin resistant Enterococcus has yet to be defined.The period of administration of antibiotics is 4 to 6 weeks.

Surgery

The surgery done for Chronic Osteomyelitis is **Sequestrectomy** and removal of the infected bone and soft tissue. The goal of surgery is complete eradication of every bit of an infection and thereby attaining a viable and vascular environment. To achieve this goal, radical debridement may be required. Inadequate debridement leads to recurrence. Simpson et al evaluated that the recurrence rate is very low with **wide resection (>5mm)** than the patients treated with marginal resection <5mm who had a 28% recurrence rate. The large dead space which is left after adequate debridement, should be managed accordingly to prevent recurrence and to avoid pathological fractures. Reconstruction of bone and soft tissue defect is done after controlling the infection.

The wound is loosely packed open with petrolatum gauze and a catheter is inserted for local application of antibiotics. If the bony defect is large, the cavity is packed with small cancellous bone grafts mixed with an antibiotic and a fibrin sealant (papineau et al (1979). The area is covered by adjacent muscle and the skin wound is sutured without tension (Lack, Bosch and Arbes, 1987). In muscle flap transfer, a large wad of muscle with intact blood supply, is laid in the cavity and the surface is covered with split- skin graft (Fitzgerald et al 1985) or Myocutaneous island flap (Yoshimura et al 1989). Recently, custom made calcium sulfate (osteoset bone voidfiller) antibiotic impregnated implants[46] is used to treat chronic osteomyelitis.

In the study conducted by A.S. Bajaj and his colleagues mentioned above, irrigation was done with appropriate antibiotics after culture and sensitivity report. It was done for 4 to 14 days and successful in 73.3 percent of cases.

About 1000 to 1500 ml of irrigating fluid was used in 24 hours. The returned fluid was cultured on 4th day and then daily. The irrigation was continued till 2 consecutive cultures of the outflow fluid became negative.

Compere [47] (1967) has recommended the use of “Alevaire” for irrigation of infection of bone and soft tissue for its mild antiseptic property as well as for its action in making penicillin effective against penicillin resistant organisms. However this last property of “Alevaire” has been refuted in invitro studies (Modellering, treth and Weinburg 1971).

As an adjuvant therapy for chronic osteomyelitis, hyperbaric oxygen therapy has been used frequently.

COMPLICATIONS

Reduced rate of growth, pathologic fracture, bone lengthening, muscle contracture, epithelioma and amyloidosis are the complications of chronic osteomyelitis.

3. OTHER TYPES OF CHRONIC OSTEOMYELITIS IN SPECIFIC CONDITIONS

a) Osteomyelitis in patients with Diabetes Mellitus or Vascular insufficiency

Patients with reduced vascular flow as in diabetes mellitus, are predisposed to osteomyelitis due to poor local tissue response. 15% of diabetes mellitus patients develop foot ulcers and 6% require hospitalization for the same [48] . The development of a skin ulcer due to neuropathy, vascular insufficiency and hyperglycemia subsequently leads to contiguous osteomyelitis.

Foot is the commonest site of infection[49] . There are several risk factors which leads to the development of ulcers in the foot in diabetic patients. All diabetic patients and patients with vascular compromise must undergo complete foot examination every year.

Diagnosis

This requires multiple modalities and a careful examination of the foot. A chronic ulcer with a surface area of more than $2cm^2$ or a positive **"Probe-to-bone test"** [50] is associated with high positive predictive value.Also complementary tests like measurement of ESR, C-reactive protein and MRI should be done.

Treatment

Broad spectrum antimicrobial therapy is required as most of these infection are **polymicrobial**[3] . Quinolones in combination with metronidazole or Clindamycin are used commonly for the treatment of osteomyelitis in patient with diabetes and peripheral vascular disease [51] . Long-term safety of fluoroquinolones has generally been good [15,48] . Latest generation quinolones such as **Moxifloxacin** have excellent activity against gram-negative and gram positive organisms and improved anaerobic activity. Depending on the level of surgical debridement and amputation, the duration of antimicrobial therapy varies from a few days to several weeks. Treatment for six weeks is appropriate [51] .

b) Tuberculous osteomyelitis

About 10% of extrapulmonary tuberculosis affects bone. Tuberculosis of spine contributes 50% of all skeletal tuberculosis cases. Most cases occurred as the result of haematogenous spread from a pulmonary source. In contrast to bacterial vertebral osteomyelitis systemic symptoms are absent. Back pain or stiffness is the common symptom. The confirmation of diagnosis is by biopsy result. CT and MRI are needed to know the extent of bony involvement which is useful for planning the treatment. In immunocompromised patients other non tuberculous Mycobacterial infection is also common. Mycobacterium fortuitum, Mycobacterium chelonae, Mycobacterium kansasii and Mycobacterium xenopi are known to cause infections. Osteomyelitis due to Mycobacterium bovis after Bacille calmette-Guerin (BCG) vaccination or intravesicular installation of Bacille calmette-

Guerin has been reported. Medical therapy alone is often curative. In certain cases, surgical debridement is required.

c) Fungal osteomyelitis

Osteomyelitis resulting from fungi is uncommon. Several observational studies and case reports have been published. Mode of spread is haematogenous (67%), direct inoculation (25%) and contiguous infection (9%) [52] . Bone lesions are most common in Blastomycosis, disseminated Coccidioidomycosis and extracutaneous Sporotrichosis, but are seen occasionally in Cryptococcosis, Candidiasis and Aspergillosis. Candida Osteomyelitis is one of the less frequent manifestations of invasive Candidiasis [53] . **Candida albicans** is the common species. Non albicans Candida species account for 35% of cases [52] . Neonates and intravenous heroin drug addicts are at risk to develop disseminated Candidiasis. In adults,the order of frequency of involvement of bones is **lumbar spine** [54] ,long bones and sternum. The diagnosis is confirmed by the isolation of candida spp from the bone and histopathological confirmation. Most cases resolve without surgery. Long term antifungal therapy is usually necessary. **Amphotericin B** and **Ketaconazole** were used commonly. Fluconazole have shown poor penetration to bone tissue.

d) Vertebral Osteomyelitis

Majority of the vertebral osteomyelitis are haematogenous in origin. Haematogenous infection of the vertebrae spreads through segmental artery. Haematogenous spread occurs via infection in skin and soft tissue, genitourinary tract infection, infective endocarditis, infected vascular sites as

well as infection of respiratory tract. In one study by Schnoring and Brock [55] , 0.2% of patient receiving antimicrobial prophylaxis developed a surgical site infection, whereas 2.8% of patients developed surgical site infection when antimicrobial prophylaxis was withheld. Infection of the disc space and contiguous vertebra also can occur postoperatively.

The most common symptom and sign of vertebral osteomyelitis are localized pain and spinal tenderness in 90% of patients.Due to nerve root compression, motor and sensory deficits, are seen in 15% of patients.

An elevation of the ESR is present in more than 90% of cases, white blood cell count is elevated in less than 50% of patient. If infective endocarditis is present, blood cultures may be positive [56] .

As with other osteomyelitis, the most common micro organisms seen in vertebral osteomyelitis are Staphylococcus aureus and Coagulase negative Staphylococci . In endemic regions Mycobacterium tuberculosis is common. In immunocompromised patients and postsurgical patients Aerobic gramnegative bacteria and Candida spp are common.

Gram negative aerobic bacteria and Candida spp are seen more commonly in IV drug abusers. Plain radiographs are not sensitive in the diagnosis of disc space infection. In a study of 41 patients with suspected spondylodiskitis, gallium scanning proved to be 100% sensitive, specific and accurate [57] . CT- guided percutaneous biopsy has a sensitivity of 50% [58] .

The aim of treatment includes eradicating the infective foci which will

relieve pain and restore neurologic function and to maintain vertebral stability. Surgical therapy is unnecessary, surgical treatment including debridement should be considered in cases with paravertebral abscess. Wit h appropriate antimicrobial medical treatment, spontaneous bony fusion between adjacent infected vertebral bodies occurs within 12 to 24 months.

e) Osteomyelitis of the craniofacial skeleton

Osteomyeliltis of the skull is truly a bony infection which is due to chronic, inadequately treated infections[59] . Van launelongue classified osteomyelitis of skull as, primary hematogenous and secondary contiguous [60] . Mandible, maxilla, frontal bone, temporal bone and skull base bones [61] are commonly affected in this type of osteomyelitis. Severe reduction of the blood flow leads to the formation of ischemic and necrotic bone [62] . The usual organisms that can be isolated are Bacteroides, microaerophilic Streptococcus spp, Peptostreptococcus, other odontogenic pathogens which affect tooth bearing bone. For planning a complete treatment, **Bone scintigraphy** is more ideal than CT. **Clindamycin** is the ideal antibiotic due to its effectiveness against Streptococci and the Anaerobes. As compared to other osteomyelitis, odontogenic infection should be treated much longer times than usual for **upto 6 months.**

PREVENTION OF CHRONIC OSTEOMYELITIS

Osteomyelitis resulting from the haematogenous spread from the focus of infection elsewhere in the body can be prevented by removing the infecting focus. For example,if the infection is due to intravenous catheter,removal of the catheter should be done and appropriate antimicrobial treatment should be

given for 6 weeks.

Osteomyelitis following any surgery is also commonly occuring now-a-days. Hence sterile aseptic surgical technique is essential for the prevention of infection and its subsequent complications.

AIM OF THE STUDY

- To study the predisposing factors associated with chronic Osteomyelitis.
- To study the causative organisms and their antimicrobial susceptibility pattern.
- To study the resistance pattern in common isolates.

MATERIALS AND METHODS

PLACE OF STUDY

The study was conducted in the Institute of Microbiology, Madras Medical College in association with Institute of Orthopaedics, Rajiv Gandhi Government General Hospital, Chennai-600 003.

Name of the Study :		Cross Sectional
Period of Study	:	Oct 2011 to Sep 2012
Sample Size	:	120

Ethical Consideration

The necessary ethical committee approval was obtained before the commencement of the study. Informed consent was obtained from the study population. All patients satisfying the inclusion criteria were documented. Patients were interviewed by structured questionnaire.

INCLUSION CRITERIA

- Patients older than 12 years.
- Patients admitted in orthopaedic wards and those attending outpatient department who satisfy one of the following six components of chronic osteomyelitis.
- Osteomyelitis in association with trauma only.
- Osteomyelitis in association with diabetes and peripheral vascular compromise.

- Clinical evidence of chronic disease. (Eg.Mycobacterium tuberculosis).
- Radiological changes suggestive of infection for 6 weeks or more.
- Formation of sequestrum or sclerosis.
- Even after treatment, persistence or relapse of infection.

EXCLUSION CRITERIA

- Patients with prosthetic orthopaedic implants devices.
- Paediatric age group (<12 years)

HISTORY

Name, age, sex, date of admission, physical examination findings, history of trauma, associated predisposing factor (diabetes mellitus, intravenous drug abuse, immunosuppression, tuberculosis) duration of illness, smoking and alcoholism were also recorded.

COLLECTION, TRANSPORT AND PROCESSING OF SAMPLES [63]

Under strict aseptic precautions samples were collected from the patients and transported immediately to the laboratory and sample processing was done.

SAMPLES COLLECTED

1) Sequestrum and fragments of excised tissue removed during surgery or curetting from infected sinuses.

2) Three swabs from the sinus tract- one for direct gram stain, acid fast

stain and KOH mount. Second for aerobic bacterial and fungal culture. Third for bedside inoculation into Robertsons cooked meat broth.

3) Pus.

COLLECTION OF SEQUESTRUM

Sequestrum obtained peroperatively were collected in a sterile container without fixative. Fragments of excised tissue removed during wound toilet or curetting from infected sinuses were also collected in a similar manner. They were homogenized in a tissue grinder [32] with a little sterile broth and subsequently treated in the same way as exudates.

COLLECTION OF SWABS

The surface of the wound was cleaned well with sterile normal saline and swabs were taken from the depth of the sinus.

COLLECTION OF PUS

Pus was aspirated from the depth of the sinus or collected directly from cavities per operatively and transported to the laboratory in a small screw-capped bottle, syringe or a sealed capillary tube [64] .

PROCESSING OF SAMPLES

1) DIRECT SMEAR EXAMINATION

Using standard laboratory techniques, pus, exudates and swabs were subjected to the following microscopic examination.

a. Gram stain: From all swabs, smears were prepared on clean glass slides and were stained with Grams stain. Here the smear was flooded with

methyl violet, waited for 1 min, washed with Grams iodine and allowed it to act for 1 min. Then washed with water, acetone was added as a decolorizing agent till no more color comes off, then washed with water and dilute carbol fuchsin was added and allowed it to act for 1 min. Then washed with water, blotted dry and examined under oil immersion objective. Presence of pus cells, yeast cells, hyphal elements, Grams reaction, size and shape of organisms were noted.

b. 10% potassium hydroxide mount [65]

A clean glass slide was taken and a large drop of KOH was placed with a pasteur pipette. A small quantity of the sample was transferred with the loop into the KOH drop. A clean cover slip was placed over the drop gently without producing air bubbles. The slide was kept at room temperature. After 20 to 30 minutes the slide was examined under microscope. Necrotic bone and tissues were allowed for overnight contact with KOH and examined on the next day.

c. Acid fast stain by Ziehl- Neelsens method [64]

Procedure

i. Place heat fixed smear on a staining rack.

ii. Filter strong carbol fuchsin on to the slide through a whatman No.1 filter paper.

iii. Soak a cotton-wool swab attached to a wire in methylated spirits.

iv. Ignite the swab in the Bunsen flame

and use to heat the slide without boiling so that they steam, leave for 3 min.

v. Repeat steps 3 and 4.

vi. Gently wash with tap water.

vii. Decolourize with 3% acid alcohol for 3 min.

viii. Gently wash with tap water.

ix. Decolourize with 20% sulphuric acid for 5 min.

x. Wash, repeat steps 9 and 10.

xi. Counterstain with loeffler's methylene blue for 30 seconds.

xii. Wash & Examine under oil immersion objective.

2) CULTURE

The samples were plated onto the following media. 5% Sheep blood agar, Chocolate agar, Mac conkey agar, Cooked-meat broth and Sabouraud's dextrose agar. All the inoculated plates except cooked meat broth were incubated at 37 ^{0}C under aerobic condition and in a carbondioxide enriched atmosphere. Plates were evaluated for growth at 24 and 48 hours and discarded after five days except Sabouraud dextrose agar which was kept for 4 weeks. Cooked meat broth was incubated at 37 ^{0}C with sterile liquid paraffin and looked for turbidity after 24 and 48 hours. If any turbidity was found in cooked meat broth, it was subcultured in Gentamycin blood agar and incubated anaerobically in gaspak at 37 ^{0}C. These anaerobic plates were examined after 72 hrs of incubation.

3. INTERPRETATION

A) INTERPRETATION OF BACTERIAL CULTURES [63,P-1443-1480-CHARTS]

I. After 24 hours of incubation, identification of bacteria was done by studying morphology of colony, gram stain, motility, catalase and oxidase tests. Single colony was taken and subjected to a battery of tests along with the controls.

- Gram staining
- Hanging drop
- Oxidase test
- Catalase test
- Coagulase test
- Phosphatase test
- Bile esculin hydrolysis
- IMVIC test
- Nitrate reduction test
- Urease test
- TSI (Triple sugar iron agar)
- Phenyl alanine deaminase test

- O-F test
- Sugar fermentation test
- LAO decarboxylases test

Oxidase test

1% tetramethyl - p - phenylene diamine dihydrochloride was prepared freshly with sterile distilled water. A filter paper circle was placed into a sterile petridish and moistened with several drops of the fresh reagent. A colony from a nutrient agar was removed with a sterile glass rod and rubbed onto the moistened filter paper along with controls. Appearance of dark purple color within 10 sec was considered as positive.

Catalase Test

Single colony from nutrient agar plate was picked with a sterile glass rod and inserted into 1ml of 3% hydrogen peroxide solution in a small clean test tube. Immediate and sustained production of gas bubbles from the colony indicate positive reaction.

Coagulase Test

This was done to detect both free and bound coagulase enzymes.

Slide coagulase test

Slide test is a rapid test to detect bound coagülase. Two drops of normal saline were placed in two circles drawn on a glass slide. Growth was taken from nutrient agar plate and emulsified into smooth suspension in two circles. Then one drop of undiluted plasma was added to one circle which was

marked as test and the other circle as control without plasma. Visible clumping within 10 - 15 sec. of mixing the plasma with the suspension was taken as positive.

Tube coagulase test

This test detects free coagulase. A small amount of the colony growth of the organism is emulsified with 0.5ml of coagulase plasma. The tube is incubated at 35 ^{0}C for 4 hours and observed for clot formation by gently tilting the tube. If no clot is observed at that time, reincubate the tube at room temperature and read again after 18 hours.

Indole Test

Organisms were suspended into Tryptophan broth and incubated at 37°C for 18-24 hrs. 15 drops of Kovac's reagent was added along the inner wall of the tube. Appearance of red ring over the surface was taken as positive.

Methyl red test

Organisms were suspended into Glucose Phosphate broth and incubated at 37°C for 48-72hrs. Then 5 drops of MR reagent was added to the broth. Appearance of red color indicated positive result.

Voges-Proskauer Test

Organisms were inoculated into Glucose Phosphate broth and incubated at 37°C for 48-72 hrs. Then 0.6m1 of 5% a naphthol was added, followed by 0.2ml of 40% KOH. The tube was gently shaken without cotton plug to expose the medium to atmospheric oxygen and was read after 10-15

minutes. Development of red color within 15 min. was taken as positive.

Citrate utilization test

This test is done to check the ability of an organism to use citrate as its whole source of carbon and energy source for growth and ammonium as nitrogen source. A saline suspension of the test organism is streaked in simmon's citrate medium and incubated for 48 hrs at 37 ^{0}c.

Blue colour and streak of growth=positive.

Original green colour and no growth=negative.

Nitrate reduction test

Nitrate broth was suspended with organisms to be tested and incubated at 37°C for 24 - 48 hrs. 5 drops of each reagent A(α naphthylamine) and reagent B (sulfanilic acid) were added to the broth. Red color developed within few minutes indicate the presence of nitrite i.e the positive reaction.

Urease test

The entire slope of the Christensen's medium was streaked with test organisms and incubated at 37°C for 24 - 96 hrs. Urease producing organisms changed the color of medium to purple pink.

Sugar fermentation tests

Sugar fermentation test media containing different sugars in the concentration of 1%, with inverted Durham's tubes were suspended with test organisms, and incubated at 37°C and observed for up to 1 week. Change of color to yellow was considered as positive (sugar was fermented). Presence of gas in Durham's tubes indicated gas production.

O-F test

Two tubes of Hugh - Leifsons test media were stab inoculated with test organisms. One tube of this pair was covered with a 1cm layer of liquid paraffin and the other tube was left open to air. Both tubes were incubated at 37°C and examined daily for up to 7 days. Appearance of yellow color in open tube and green color in covered tube, indicated oxidative utilization of the sugar.

Triple Sugar Iron test

The organism was stabbed into butt and streaked onto the surface of slant, incubated at 37°C overnight. The next day change of color, H_2S production and presence of gas were noted.

Phenyl alanine deaminase test

The medium containing phenyl alanine deaminase was suspended heavily with organism to be tested and incubated at 37°C overnight. Next day few drops of 10% solution of ferric chloride was allowed to run down over the growth. Appearance of apple green color in the slope indicated positive test.

Phosphatase test

Organism was grown on phenolphthalein diphosphate agar (consist of l000ml of nutrient agar and l0ml of 1% aqueous solution of sodium phenolphthalein diphosphate) at 37°C overnight. Next day few drops of liquor ammonia was poured on lid and plate was inverted over the lid. Colonies turned to bright pink, from yellow color within a few minutes, due to

liberation of free phenolphthalein by the action of phosphatase. This was considered as positive.

Bile Esculin Hydrolysis

Organism was streaked on the surface of bile esculin slant and incubated over night. If the organism hydrolyses bile esculin ,slant would be turned black. This test was used to confirm gram positive cocci in pairs as Enterococci.

LAO Decarboxylases Test

This test is based on the ability of bacteria to decarboxylate an aminoacid to the corresponding amine with liberation of carbondioxide. The production of decarboxylases is induced by a low pH which occurred due to fermentation of glucose and as a result of the action of decarboxylases ,the pH is raised due to the production of amines. The medium was inoculated with a straight wire through the paraffin layer .Incubated and read daily for 4 days. Appearance of violet colour indicate positive test.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antibiotic susceptibility testing was performed by the Kirby bauer method on Mueller Hinton agar (Himedia) according to CLSI guidelines[66] . The diameters of zones of inhibition were interpreted according to CLSI standards for each organism. Media and discs were tested for quality control using standard strains.

The following standard strains were used

1) Staphylococcus aureus- ATCC 25923

2) Escherichia coli- ATCC 25922

3) Pseudomonas aeruginosa- ATCC 27853

For gram positive cocci, the following antibiotics were included in the antimicrobial sensitivity testing (Himedia)

Antibiotic	**Disc content**	**Inhibition Zone in mm**		
		Resistance	**Intermediate**	**Sensitive**
Amikacin	30□g	14	15-16	17
Ciprofloxacin	5□g	15	16-20	21
Cotrimoxazole	1.25-23.75□g	10	11-15	16
Chloramphenicol	30□g	12	13-17	18
Penicillin	10 Units	28	–	29
Rifampin	5□g	16	17-19	20
Erythromycin	15□g	13	14-22	23
Oxacillin	1□g	10	11-12	13

For gram negative bacilli, the following antibiotics were included in the antimicrobial sensitivit y testing (Himedia)

Antibiotic	Disc content	Inhibition Zone in mm		
		Resistance	Intermediate	Sensitive
Amikacin	30□g	14	15-16	17
Ceftazidime	30□g	14	15-17	18
Cefotaxime	30□g	14	15-17	18
Ciprofloxacin	5□g	15	16-20	21
Gentamicin	10□g	12	13-14	15
Imipenem	10□g	13	14-15	16
Ofloxacin	5□g	12	13-15	16
Tetracycline	30µg	14	15-18	19
Piperacillin/ Tazobactum	100/10□g	17	18-20	21

Procedure of Kirby-Bauer Disc Diffusion Test

1) With a wire or a loop touched the surface of 5 similarly appearing colonies on an agar plate culture. Transferred the growth to a tube containing a suitable broth medium.

2) Allowed the culture to incubate at 35 ^{0}C until it matches the turbidity of standard.

3) Dipped a sterile non-toxic cotton swab into the inoculum suspension and rotated the swab several times with firm pressure on the inside wall of the tube to remove excess of fluid.

4) Inoculated the dried surface of a Mueller Hinton agar plate that has been brought to room temperature by streaking the swab 3 times over

the entire agar surface rotating the plate approximately 60 degrees to ensure an even distribution. Replaced the lid of the dish. Allowed 3 to 5 minutes but no longer than 15 minutes for the surface of the agar to dry before adding the antibiotic discs.

5) Appropriate antimicrobial disc was placed on the surface of the agar using forceps.

6) Plate was incubated at 37 ^{0}c overnight.

7) After overnight incubation, zone diameters was measured in mm from the edge of the disc to the zone edge with a ruled template on the agar surface

DETECTION OF □ LACTAMASE ENZYMES IN GRAM NEGATIVE BACILLI

Extended spectrum □ lactamases (ESBL's)

ESBL's are classified under in Bush class A □ lactamases which are capable of hydrolyzing penicillins- oxyiminocephalosporins and monobactams (Aztreonam) and inhibited by □ lactamase inhibitors (clavulanic acid, sulbactum and Tazobactum) but have no detectable activity against cephamycins or carbapenems (Imipenem, Meropenem).

ESBL Detection methods

Screening Method [67]]

Antibiotic	For Possible ESBL Producing Stains
Aztreonam 30□g	≤ 27mm
Cefotaxime 30□g	≤ 27 mm
Ceftazidime 30□g	≤22 mm
Ceftriaxone 30□g	≤ 25mm

Double Disk diffusion synergy test

24 hour young culture was used for this test. 3 to 4 colonies from 24 hr culture were inoculated into 5 ml of nutrient broth to match 0.5 Macfarland turbidity standard. Lawn culture of the test organism should be made on MHA plate. Two discs Ceftazidime and Ceftazidime in combination with clavulanic acid were placed. The plate is incubated at 35_0 C for 16-18 hours.

Interpretation

A $\geq$5mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone confirms an ESBL producing organism[68,66] .

Phenotypic confirmatory double disk test (PCDDT)

In this method a lawn culture of test organism on to a MHA plate was performed. Augmentin disc was placed in the centre of the plate and a disk containing one of the oxyimino lactam antibiotics was placed 30mm from centre to centre from augmentin disk. The test organism was considered to produced ESBL, if the zone size around the test antibiotic disc increased towards the augmentin disk [69] .

The sensitivity of the test could be increased by reducing the distance between the disc to 15mm or 20mm, also by using more than one oxyimino lactam antibiotic.

ESBL detection by E test strip

This combines both the principles of dilution and diffusion techniques. E strip is a thin non porous plastic strip 5mm wide and 60mm long. It carries

two shorter gradients aligned in opposing directions on a single strip. One end generates a stable concentration gradient of the one of the oxyimino cephalosporins (eg ceftazidime), while the other end generates a gradient of cephalosporin + clavulanic acid (4□g/ml). When applied to an inoculated agar plate inhibition ellipse may be seen on the both ends of the strip. MIC is interpreted as the point of intersection of the inhibition ellipse with E test strip edge. Ratio of cephalosporin MIC and cephalosporin clavulanic acid MIC >8 indicates positive result.

DETECTION OF METHICILLIN RESISTANCE IN STAPHYLOCOCCUS AUREUS

Disc diffusion Method

Media- MHA

Antibiotic disc- Cefoxitin disks 30□g

QC Strain – ATCC S.aureus 25923 was used.

Procedure

Inoculum Preparation

Colonies isolated from agar culture plate were suspended directly into broth or physiological saline (0.85% Nacl, vortexed to reach 0.5 McFarland (10^8 CFU/ml).

A lawn culture of the Staphylococcus aureus was made on the MHA plate and the cefoxitin disks(30µg) were applied.

Incubated at 35 ^{0}C for 24 hrs in an ambient air.

The diameter of the zone of inhibition around the discs was measured.

According to CLSI criteria,2011,with 30µg cefoxitin disks [70,66 -supp ta b-2C-S4] ,

Diameters of <21mm= Resistant to oxacillin(MRSA)

≥22mm = Susceptible to oxacillin(MSSA).

No intermediate category.

DETERMINATION OF MIC

The following are the different methods of detecting MIC of an antibiotic against a specific bacterial isolate.

1) Broth dilution method

a) Macrobroth method

b) Microbroth method

2) Agar dilution method

3) Using E strip

4) Using Hi-comb method

MIC of vancomycin against Staphylococcus aureus was determined by Macro Broth dilution method.

MIC of cefotaxime against Klebsiella pneumoniae was determined using Hi-Comb method.

Procedure of Hi-comb method

A lawn culture of the test organism was made on a MHA plate Hi-Comb Strips were applied with the MIC scale facing upward. The strip should be in complete contact with the agar surface. The agar plate was inverted and incubated at 35 ^{0}C for 24hrs. The method combines the principle of dilution

and diffusion. The E test MIC was read where the edge of the inhibition ellipse intersects the MIC scale on the strip

Minimum Inhibitory Concentration (MIC) for detecting vancomycin resistance

1) Cation adjusted Mueller Hinton Broth (PH 7.2-7.4) was used.

2) **Preparation of stock antibiotic solution** [71]

Antibiotic stock solution was prepared using the formula.

$$\frac{1000}{P} \times V \times C = W$$

Where P= Potency of the antibiotic in relation to the base (For vancomycin, P= 950/1000mg Himedia).

V = Volume of the stock solution to be prepared (10ml).

C = Final concentration of the antibiotic solution (1024 □g/ml).

W = Weight of the antibiotic to be dissolved in the volume V.

3) Method of preparing dilution of antibiotics

□ Sterile test tubes were arranged in two rows in the rack (1 row for the test and another one is for ATCC control).

□ Transferred 2ml of MH broth to the sterile container containing the working stock solution (128 □g/ml concentration). From this 1ml was transferred to the first tube in each row.

□ 2ml of MH broth was added to the 2ml of the diluted antibiotic in the

sterile container, mixed well and 1ml was transferred to second tube in each row.

- Repeated this procedure till the 8 th tube.
- One ml of the antibiotic was kept for control.

4) Inoculum preparation for the test and ATCCcontrol

- 9.9ml of MH broth was taken in a sterile container.
- 0.1ml of 0.5 Mcfarland tubidity matched test organism was added.
- After mixing well, 1ml of inoculum was transferred to each tube containing antibiotic dilutions and also to the control tube.
- The procedure was repeated for ATCC control stain.

The tubes were incubated at 37 ^{0}C for 16-18 hrs. After 16-18 hrs, the tubes were taken out and MIC was read. The lowest concentration of the antibiotic in which there is no visible growth was taken as MIC of Vancomycin for the test organism. The above procedure was repeated for ATCC S.aureus, and MIC was interpreted.

B.INTERPRETATION OF FUNGAL CULTURE

Samples were inoculated onto two SDA slants and were incubated at two different temperatures, 25 ^{0}C and 35 ^{0}C. These slants were inspected daily during the first week and twice weekly during the next three weeks for growth.

Macroscopic Appearance

Cream colored, smooth or wrinkled with mycelial fringe and pasty colonies within 3-4 days.

Microscopy

Gram stain and LCB mount were prepared from the colonies.

Gram Stain Gram positive ovoid budding yeast cells approximately 4-8 □m in size with pseudohyphae is suggestive of candida spp.

LCB Presence of yeast cells and pseudohyphae.

Germ tube test *[65]*

Germ tube test is used for presumptive identification of Candida albicans. It is a rapid screening test where the production of germ tubes with in two hours is considered as growth of Candida albicans.

Make a very light suspension of the test organism in 0.5ml of sterile serum (pooled human serum or fetal calf serum). The optimum inoculum is 10^{5}-10^{6} cells per ml. Incubate at 37^{0}C for exactly two hours. Observed under microscope for production of germ tube. Germ tubes represent initiation of hyphal growth, arising directly from the yeast cell. They have parallel walls at their point of origin and are not constricted. To record a positive, about 30% of the cells should show germ tube production.

Chrom Agar Media *[72]*

It is a rapid, plate based test for the simultaneous isolation and identification of various Candida species. These media are based on direct

detection of specific enzymatic activities by adding multiple chemical dyes i.e. substrates of fluorochromes to media. Candida species are differentiated by color as a result of biochemical reactions.

The CHROM agar media shows following colors of colories at 30 ^{0}C for 48 to 72 hours.

C.albicans	Light Green
C.dubliniensis	Dark Green
C.glabrata	Pink to Purple
C.krusei	Pink
C.parapsilosis	Cream to Pale Pink
C.tropicalis	Blue with Pink halo

Sugar fermentation

Biochemical tests like sugar fermentation was done for identification of yeast isolate. Glucose, Maltose, sucrose, Lactose, Galactose and Trehalose sugars (2%) were used.

Determination of MIC by microbroth dilution method *[65]*

As per the guidelines of CLSI, the test was performed. MIC of water soluble drug Fluconazole and water insoluble drugs Amphotericin-B, Itraconazole and Voriconazole were determined.

The test consisted of following steps

1. Preparation of antifungal stock solutions.

2. Preparation of inoculum.

3. Test procedure.

For water insoluble drugs, dimethyl sulfoxides (DMSO) was the solvent used.

Media used- RPMI 1640. Varying concentrations of the drugs were tested.

Amphotericin B	0.0313 to 16□g/ml
Fluconazole	0.125 to 64□g/ml
Itraconazole and Voriconazole	0.0313 to 16□g/ml

Incubation period – 48 hours.

ATCC candida albicans ATCC 90028 was used for quality control of the test. The broth microdilution test was performed by using sterile, disposable, multiwell microdilution plates (96U- shaped wells). 100□l of varying drug concentrations was dispensed in each rows from 1 to 10. 100□l of inoculum was also dispensed. Incubated at 35 ^{0}C for 48 hours. MIC was interpreted, as the lowest concentration in which the well was clear in case of Amphotericin B. For Azoles, it was interpreted as the lowest concentration in which there was a prominent decrease in turbidity (50% inhibition in growth as determined spectrophotometrically).

C.CULTURE FOR MYCOBACTERIUM TUBERCULOSIS

All the samples were screened for the presence of acid fast bacilli by Ziehl Neelsen method of acid fast staining. Few samples were sent to Tuberculosis Research Centre, Chetpet for culture and sensitivity.

RESULTS

This study was conducted in the Institute of Microbiology in association with the Institute of Orthopaedics, Rajiv Gandhi Government General Hospital, Chennai-600 003.

TABLE-1: AGE AND SEX DISTRIBUTION

Age (Years)	No. of Patients		Total n =120
	Male n =97	Female n =23	
<20	18 (18.5%)	5 (21.7%)	23 (19.1%)
21-30	21 (21.6%)	7 (30.4%)	28 (23.3%)
31-40	25 (25.7%)	5 (21.7%)	30 (25%)
41-50	18 (18.5%)	2 (8.6%)	20 (16.6%)
51-60	9 (9.2%)	4 (17.3%)	13 (10.8%)
61-70	6 (6.1%)	0	6 (5%)

Chi square: 4.471 **p= 0.484** [not significant]. The **mean age** of male is **35.94.** The **mean age** of female is **31.78.** Out of 120 patients, 30 patients belonged to age group 31-40 years (25% of total cases), 28 patients belonged to the age group 21-30 years (23% of total cases). In all age groups, males were commonly affected because they were more prone to accidents than females as they do outdoor work, construction work and high altitude work. In this study, 97 were males and 23 were females.

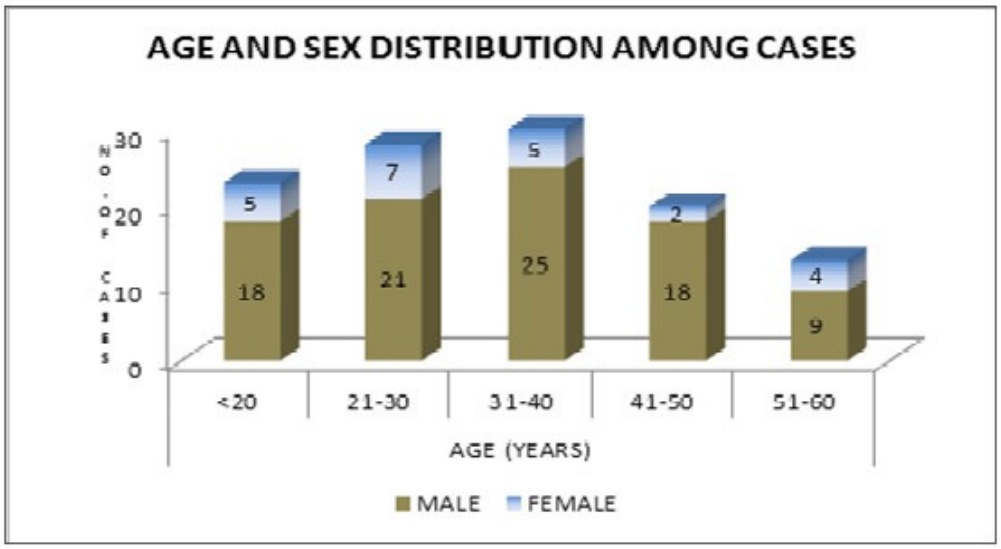


TABLE-2: DURATION OF ILLNESS

Duration in Months	No. of Patients n =120	Percentage
2-6	19	15.8
7-12	48	40
13-24	43	35.8
25-36	10	8.3

Most patients (40%) had duration of illness between 7-12 months. This was followed by the patients (35.8%) who had duration of illness between13-24 months.

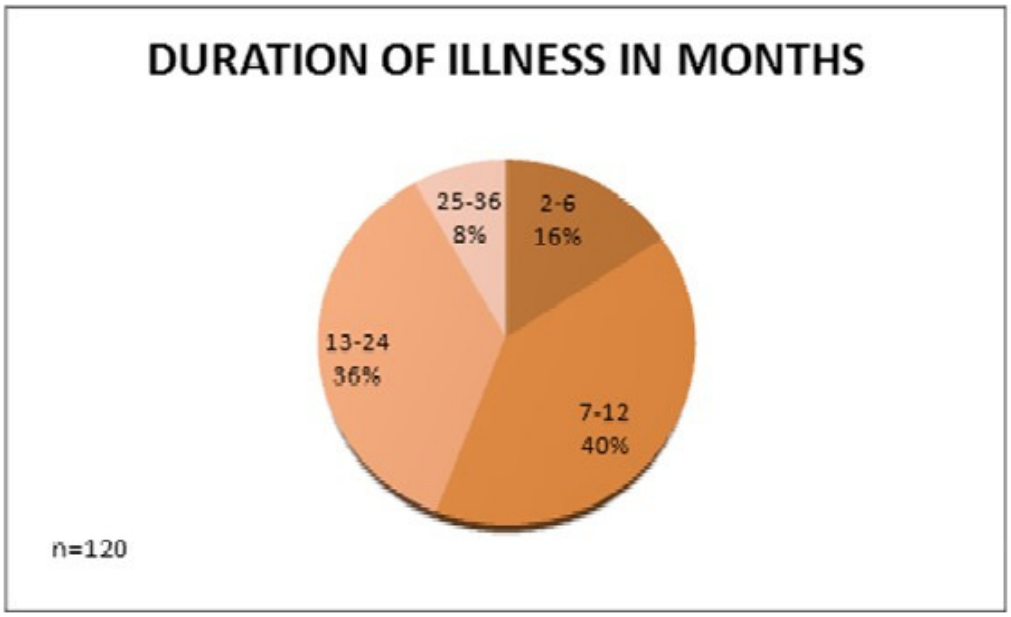


TABLE-3: CORRELATION OF SEX AND DURATION OF ILLNESS

	SEX	n	Mean	Std.Std. Error Significance Deviation of mean		
DURATION ACTUAL MONTHS	M	97	14.03	7.555	.767	P=0.574
	F	2	13.00	9.200	1.918	

The mean duration of illness in males is 14.03.P=0.574[not significant]. The mean duration of illness in females is 13.00.

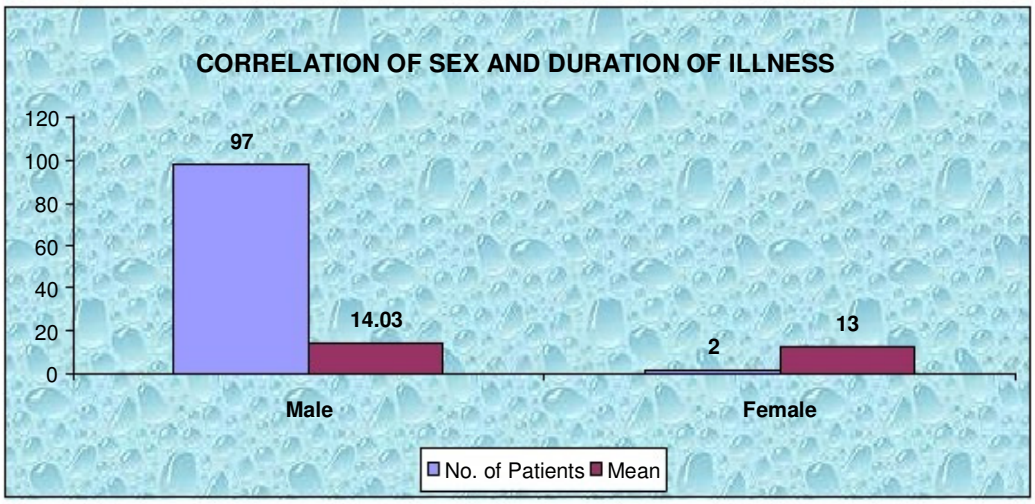


TABLE-4: CORRELATION OF AGE AND DURATION OF ILLNESS

Groups	n	Mean	Std. Deviation
1	19	29.47	16.473
2	48	30.33	10.041
3	43	39.02	15.056
4	10	52.30	12.962
Total	120	35.14	14.772

Groups :1-duration of illness 2-6 months

2-duration of illness 7-12 months

3-duration of illness 13-24 months

4-duration of illness 25-36 months.

P = 0.000<0.001 . The mean age level in the duration of illness is statistically significant. Among the duration of illness, group with largest duration had age mean level as 52.30 , which is significantly an elevated age mean level than other duration group.

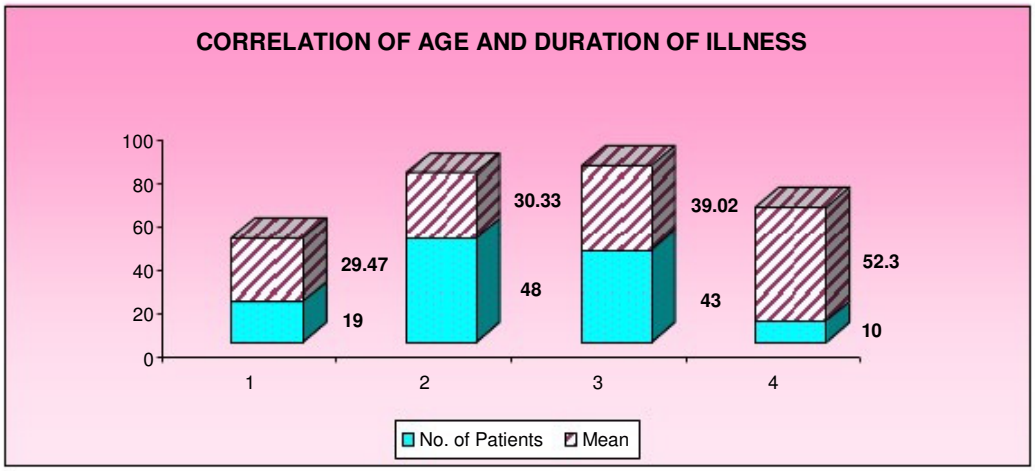


TABLE-5: PREDISPOSING FACTORS

Duration in Months	No. of Patients n =120	Percentage	Lower confidence limits in percentage	Upper confidence limits in percentage
Compound fracture due to road side accidents	61	50.8%	41.92	59.71
Post Surgical	24	20.1%	13.25	28.28
Diabetes mellitus with vascular insufficiency	17	14.1%	8.474	21.71
Smoking/ Alcoholism	13	10.8%	5.896	17.81
Haematogenous	5	4.1%	1.366	9.456

Among 120 cases of chronic osteomyelitis, 61 patients developed the infection as a result of compound fracture due to road side accidents and trauma (50.8%). 24 cases (20.1%) were due to post surgical wounds. 17 cases (14.1%) had diabetes mellitus as the predisposing factor. 13 cases (10.8%) had smoking/ alcoholism as the predisposing factor. 5 patients (4.1%) acquired the infection of bone due to haematogenous spread of organisms.

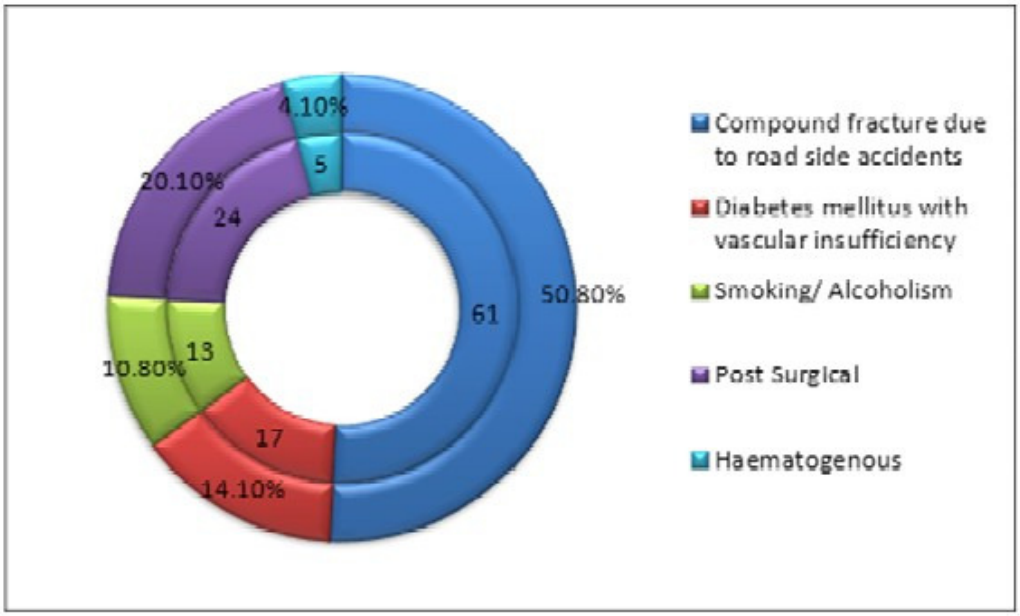


TABLE-6: SITE OF INFECTION

Bone	No. of Patients n =120	Male n =97	Female n =23	Percentage
Tibia	53	43	10	44.2
Femur	33	24	9	27.5
Metatarsal	6	6	0	5
Humerus	5	5	0	4.1
Calcaneum	4	4	0	3.3
Dorsal Spine	3	2	1	2.5
Frontal Bone	3	1	2	2.5
Fibula	2	2	0	1.6
Radius	2	2	0	1.6
Rib	2	2	0	1.6
Sacrum	2	2	0	1.6
Sternum	2	2	0	1.6
Maxilla	1	1	0	0.8
Clavicle	1	1	1	0.8
Femur /Tibia	1	1	0	0.8
Total	120	97	23	100

p= 0.545 . No statistical significance was found between the male and female with respect to different bone sites.

The commonest bone infected was tibia in 53 patients (44%), out of which 43 were males and 10 were females. Femur was the second common

bone infected in 33 patients (29%). Out of 33,24 were males and 9 were females. Other bones of lower extremities like metatarsals, calcaneum and fibula were affected in the order of 6 patients (5%), 4 patients (4%) and 2 patients (2%) respectively. Only males got infected in these bones. Bones of the upper extremities like Humerus and Radius were infected in 5 patients (4.1%) and 2 patients (1.66%) respectively. They were males. 3 patients developed infection in frontal bone. Among them 2 were females and one was male. One male patient had infection in maxilla. Bones of the axial skeleton like thoracic vertebrae, sacrum, sternum and clavicle were involved in 3 patients (2.5%), 2 (1.66%), 2 (1.66%), and 1 (0.8%) respectively. Out of 3 patients who got infected in thoracic vertebrae 2 were male and one was female. Only males were affected with the involvement of sacrum, sternum and clavicle.

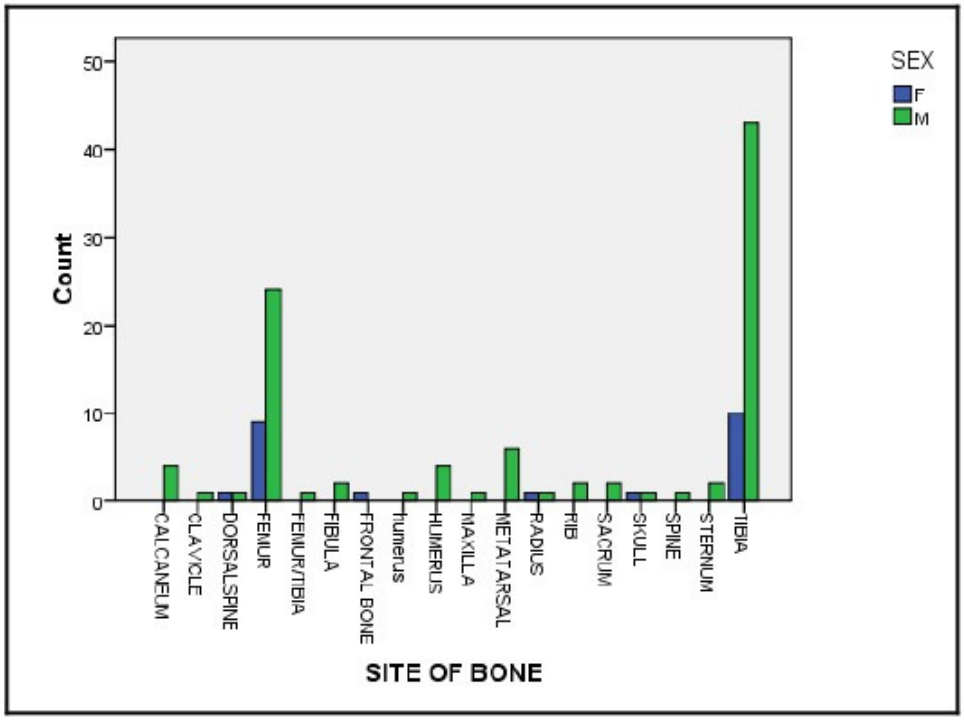


TABLE-7-SITE OF BONE*AGE GROUP CROSSTABULATION

	Age Group						
	1	**2**	**3**	**4**	**5**	**6**	**Total**
CALCANEUM	0	2	1	1	0	0	4
	.0%	1.7%	.8%	.8%	.0%	.0%	3.3%
CLAVICLE	0	0	0	0	1	0	1
	.0%	.0%	.0%	.0%	.8%	.0%	.8%
DORSALSPINE	0	2	0	0	0	0	2
	.0%	1.7%	.0%	.0%	.0%	.0%	1.7%
FEMUR	10	7	10	3	2	1	33
	8.3%	5.8%	8.3%	2.5%	1.7%	.8%	27.5%
FEMUR/TIBIA	0	0	0	1	0	0	1
	.0%	.0%	.0%	.8%	.0%	.0%	.8%
FIBULA	0	0	2	0	0	0	2
	.0%	.0%	1.7%	.0%	.0%	.0%	1.7%
FRONTAL BONE	0	0	0	1	0	0	1
	.0%	.0%	.0%	.8%	.0%	.0%	.8%
HUMERUS	0	3	1	1	0	0	5
	.0%	2.5%	.8%	.8%	.0%	.0%	4.1%
MAXILLA	0	0	0	1	0	0	1
	.0%	.0%	.0%	.8%	.0%	.0%	.8%
METATARSAL	1	0	2	0	1	2	6
	.8%	.0%	1.7%	.0%	.8%	1.7%	5.0%
RADIUS	1	1	0	0	0	0	2
	.8%	.8%	.0%	.0%	.0%	.0%	1.7%
RIB	0	1	0	0	0	1	2
	.0%	.8%	.0%	.0%	.0%	.8%	1.7%
SACRUM	0	0	2	0	0	0	2
	.0%	.0%	1.7%	.0%	.0%	.0%	1.7%
SKULL	0	1	1	0	0	0	2

	Age Group						
	1	**2**	**3**	**4**	**5**	**6**	**Total**
	.0%	.8%	.8%	.0%	.0%	.0%	1.7%
SPINE	0	1	0	0	0	0	1
	.0%	.8%	.0%	.0%	.0%	.0%	.8%
STERNUM	0	0	0	2	0	0	2
	.0%	.0%	.0%	1.7%	.0%	.0%	1.7%
TIBIA	11	10	11	10	9	2	53
	9.2%	8.3%	9.2%	8.3%	7.5%	1.7%	44.2%
Total	23	28	30	20	13	6	120
	19.2%	23.3%	25.0%	16.7%	10.8%	5.0%	100.0%

p= 0.140. There is no statistical significance exists among different age groups with respect to the bone site.

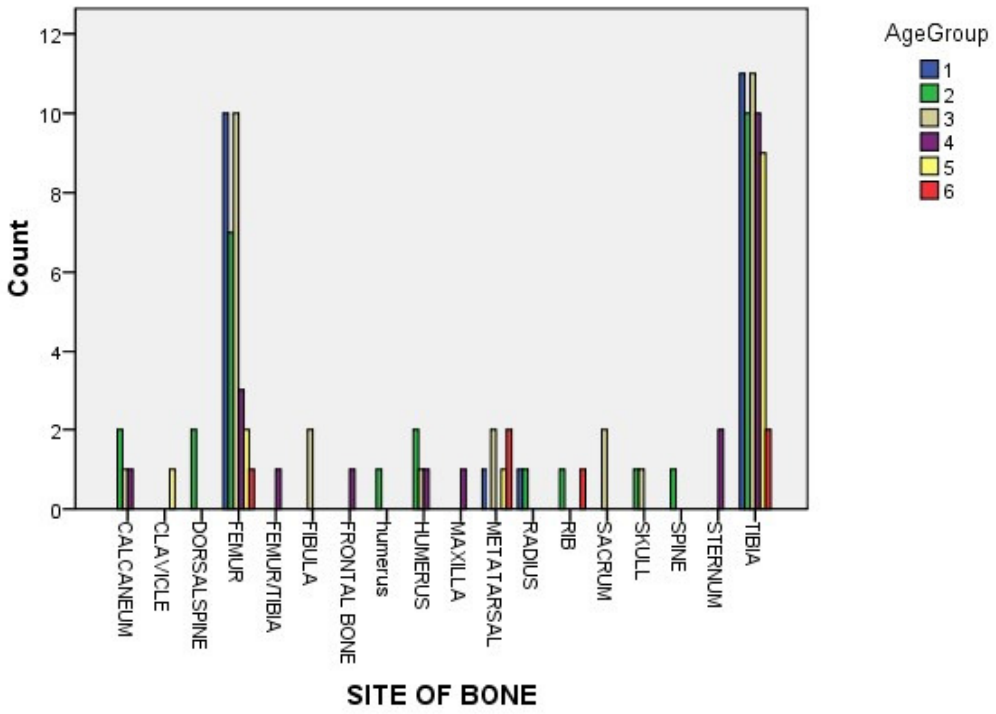


TABLE-8: SAMPLES COLLECTED FROM THE STUDY GROUP

Samples	Number n=120	Percentage	LCL in percentage	UCL in percentage
Discharge from sinus	57	47.5	38.31	56.82
Sequestrum	41	34	25.76	43.38
Intraoperative collection of pus/tissue fluids	22	18.3	11.86	26.43

LCL - lower confidence limit, UCL-upper confidence limit

From the above table, it was found that 57 patients (47.5%) had discharge from sinuses as the presenting symptom. The other patients had pain, low grade fever and swelling as the presenting symptoms. Among the samples collected from them, 57 were collected as discharge from sinuses, 41 (34.1%) as sequestrum and 22 (18.3%) as intraoperative collection of pus.

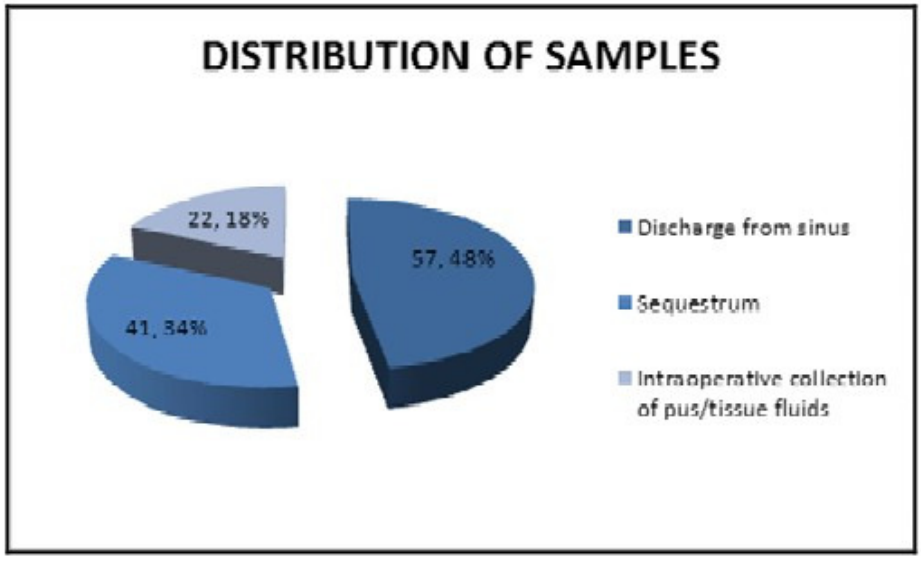


TABLE-9: CULTURE POSITIVITY

Culture	Number n =120	Percentage	Lower confidence limit in percentage	Upper confidence limit in percentage
Positive	100	83.3	75.44	89.51
No Growth	20	16.6	10.49	24.56

Among 120 cases studied, culture positivity was seen in 100 patients (83.3%). Out of 100, 92 (76.6%) were grown as pure culture (monomicrobial). 9 (7.5%) showed mixed growth (polymicrobial), 20 (16.6%) showed no growth.

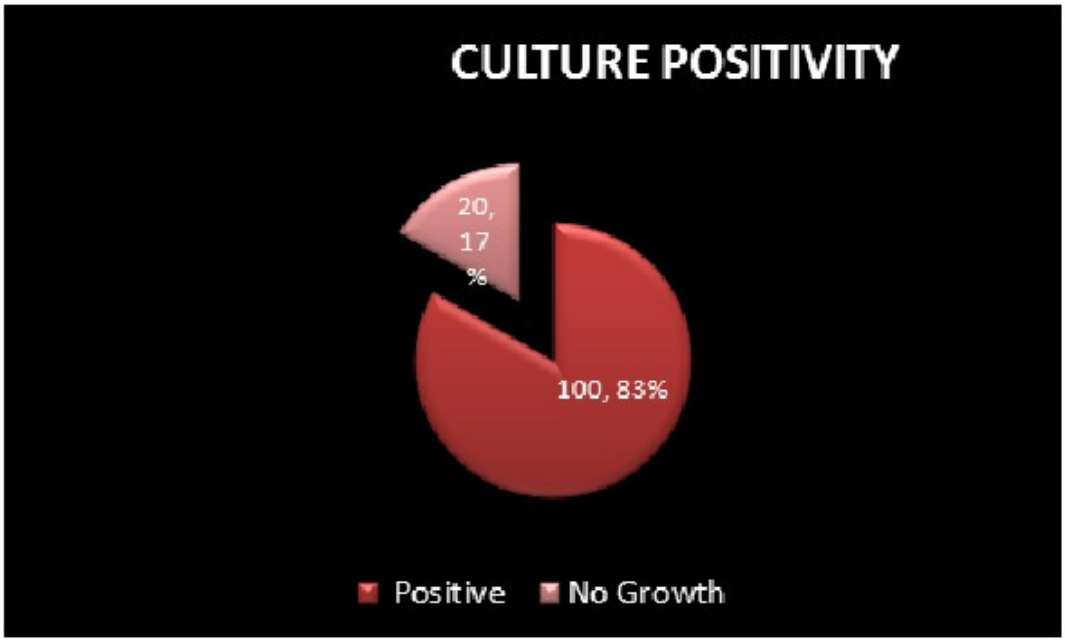


TABLE-10: CORRELATION BETWEEN TYPE OF SPECIMEN COLLECTED AND TYPE OF PATHOGENS ISOLATED

Type of Pathogen	Swab n=57	Sequestrum/ Intraoperative collection of pus and tissue fluids n=63	Total n=120
Monomicrobial	33(57.8%)	59(93.6%)	92
Polymicrobial	7 (12.2%)	1(1.5%)	8
No Growth	17(29.8%)	3(4.7%)	20

Chi-square: 21.4 **P= 0.0000253<0.0001** significant.

Polymicrobial growth was seen more in swabs when compared to sequestrum and intraoperative collections. Hence sequestrum or intraoperative collections are the ideal samples for culture.p value also showed statistical significance.

Pathogens isolated were monomicrobial in 92 cases, polymicrobial in 8 cases. Of the 63 sequestrum/ intraoperative collection of pus and fluids, 59 were monomicrobial and only one was polymicrobial. Mycobacterium tuberculosis and Candida tropicalis were isolated only from sequestrum samples. Of the 57 swabs, 33 were monomicrobial 7 were polymicrobial. Though monomicrobial infection was the commonest type in both samples, an increased number of polymicrobial infection was noted only in swabs.

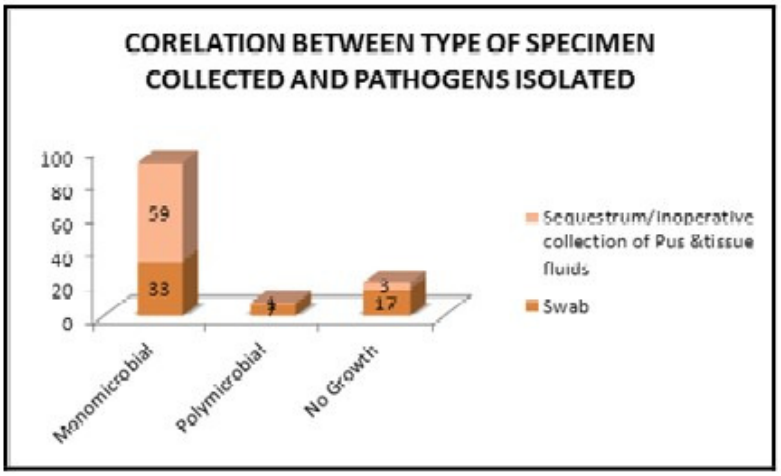
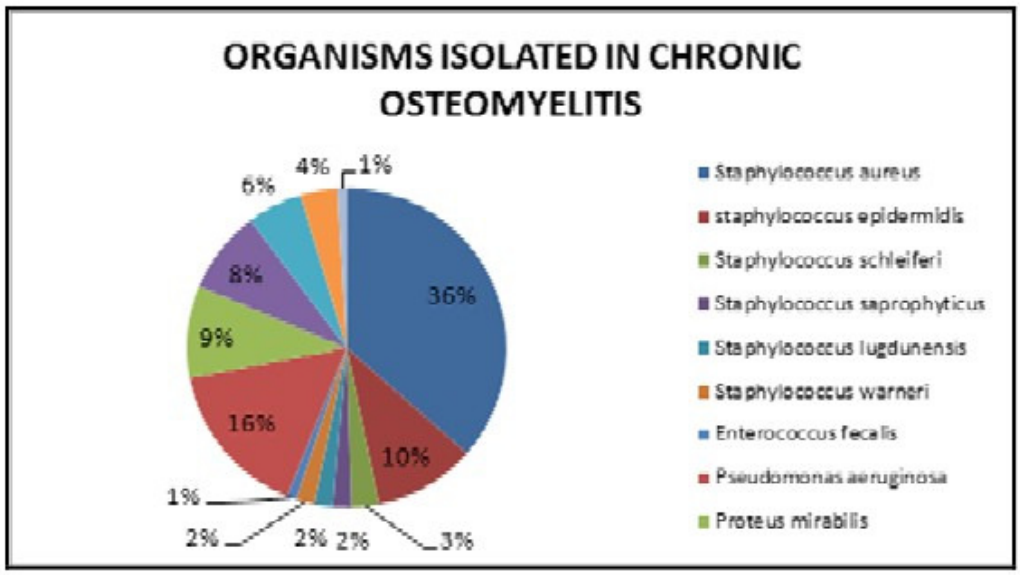


TABLE-11: ORGANISMS ISOLATED IN CHRONIC OSTEOMYELITIS

Organisms	Number	Percentage
Aerobic bacterial isolates n =106		
Gram Positive Cocci		
Staphylococcus aureus	39	36.7
Staphylococcus epidermidis	11	10.3
Staphylococcus schleiferi	3	2.8
Staphylococcus saprophyticus	2	1.8
Staphylococcus lugdunensis	2	1.8
Staphylococcus warneri	2	1.8
Enterococcus fecalis	1	1
Gram Negative Bacilli		
Pseudomonas aeruginosa	17	16
Proteus mirabilis	10	9.4
Klebsiella pneumoniae	9	8.4
Escherichia coli	6	5.6
Acinetobacter baumanii	4	3.7
Acid fast bacilli *n=1*		
Mycobacterium tuberculosis	1	1.08
Fungal *n=1*		
Candida tropicalis	1	1.08

The above table shows, 56.6% of isolation of GPC, 43.3% of isolation of GNB, one acid fast bacillus *Mycobacterium tuberculosis* and one yeast *Candida tropicalis* was also isolated in this study.


ORGANISMS ISOLATED IN CHRONIC OSTEOMYELITIS
36%
10%
3%
2%
2%
2%
1%
16%
9%
8%
6%
4%
1%
Staphylococcus aureus
staphylococcus epidermidis
Staphylococcus schleiferi
Staphylococcus saprophyticus
Staphylococcus lugdunensis
Staphylococcus warneri
Enterococcus fecalis
Pseudomonas aeruginosa
Proteus mirabilis

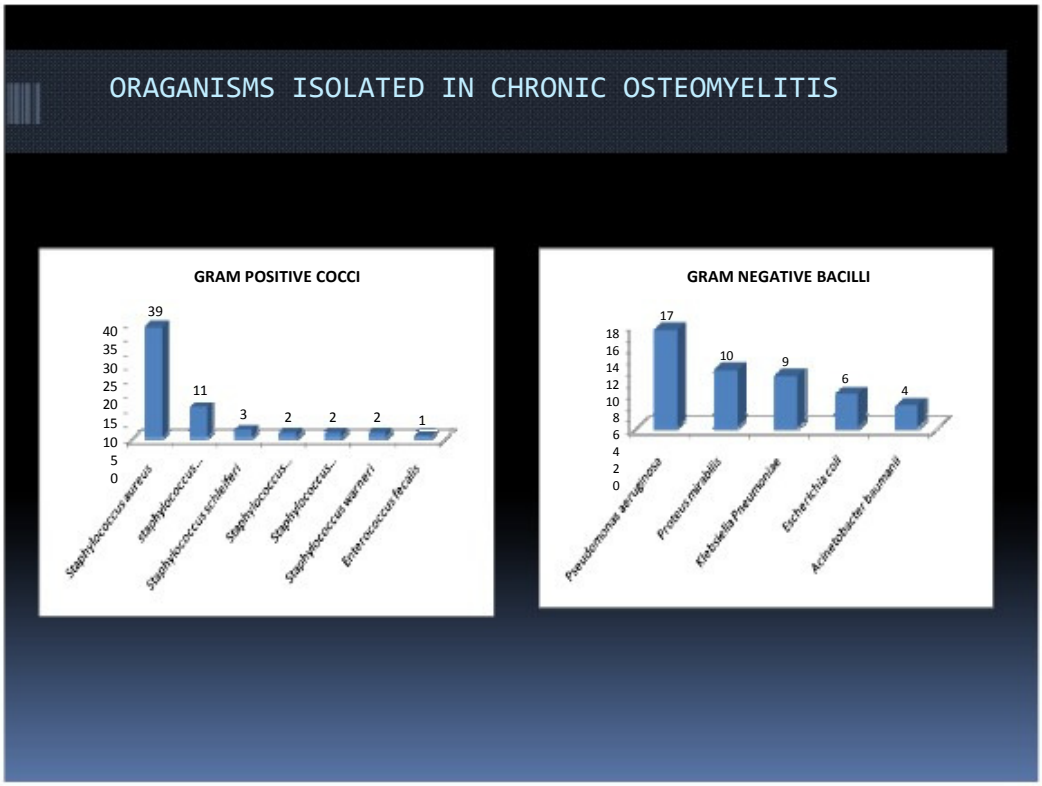

ORAGANISMS ISOLATED IN CHRONIC OSTEOMYELITIS
GRAM POSITIVE COCCI
39
11
3
2
2
2
1
Staphylococcus aureus
staphylococcus...
Staphylococcus schleiferi
Staphylococcus...
Staphylococcus...
Staphylococcus warneri
Enterococcus fecalis
GRAM NEGATIVE BACILLI
17
10
9
6
4
Pseudomonas aeruginosa
Proteus mirabilis
Klebsiella Pneumoniae
Escherichia coli
Acinetobacter baumanii

TABLE-12: COMBINATION OF BACTERIAL ISOLATES IN MIXED INFECTIONS

Organisms	No of patients n=8	Percentage
Staphylococcus aureus and Pseudomonas aeruginosa	3	37.5%
Staphylococcus schleiferi and Enterococcus fecalis	1	12.5%
Staphylococcus epidermidis and Klebsiella Pneumoniae	1	12.5%
Proteus mirabilis and Escherichia coli	3	37.5%

Staphylococcus aureus ,Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli were commonly isolated in mixed infections.3 out of 39 isolates of *Staphylococcus aureus,3* out of 17 isolates of *Pseudomonas aeruginosa,3* out of 10 isolates of *Proteus mirabilis,3* out of 6 isolates of *Escherichia coli* were isolated in mixed infections.one out of 11 isolates of *S.epidermidis,1* out of 3 isolates of *S.schleiferi* and 1 out of 9 isolates of *Klebsiella pneumoniae* were also isolated in mixed infections.

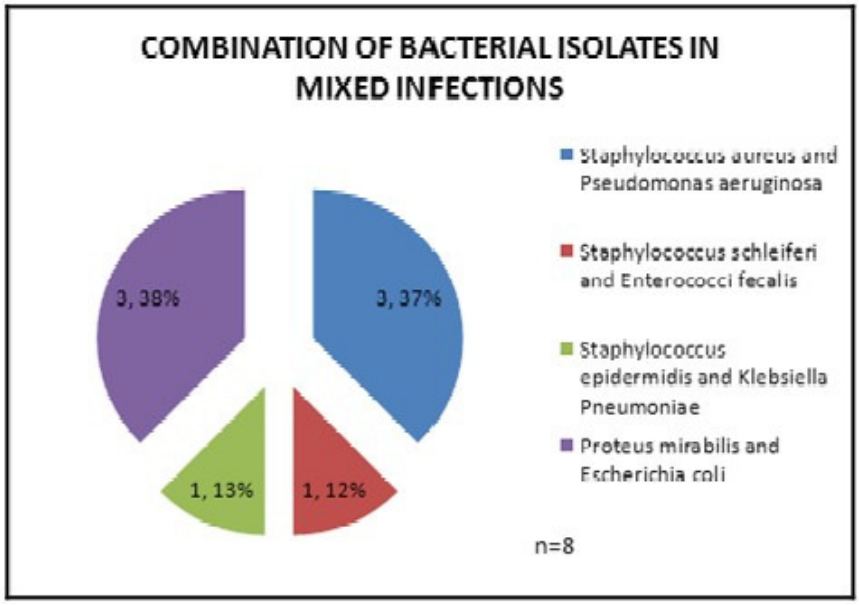


TABLE-13: ANTIMICROBIAL SENSITIVITY PATTERNS OF GRAM POSITIVE COCCI (GPC)

Antibiotics	Staphylococcus aureus n =39		Staphylococcus epidermidis n =11		Staphylococcus schleiferi n =3		Staphylococcus lugdunensis n =2		Staphylococcus saprophyticus n =2		Staphylococcus warneri n =2		Enterococcus fecalis n=1	
	Isolate	%	Isolate	%	Isolate	%`	Isolate	%	Isolate	%	Isolate	%	Isolate	%
Amikacin	25	64%	8	72.7%	3	100%	1	50%	1	50%	1	50%	1	100%
Ciprofloxacin	10	25.6%	3	27.2%	0	0	1	50%	1	50%	1	50%	0	0
Chloramphenicol	25	64.1%	6	54.5%	1	33.3%	2	100%	1	50%	1	50%	1	100%
Cotrimoxazole	23	58.9%	3	27.27%	1	33.3%	1	50%	0	0	1	50%	0	0
Cephalexin	21	53.8%	3	27.27%	1	33.3%	1	50%	0	0	0	0	0	0
Erythromycin	25	64.1%	2	18.18%	1	33.3%	1	50%	0	0	0	0	1	100%
Penicillin	20	51.2%	5	45.4%	0	0	1	50%	0	0	0	0	0	0
Rifampin	39	100%	11	100%	3	100%	2	100%	2	100%	2	100%	1	100%
Vancomycin	38	97.4%	11	100%	3	100%	2	100%	2	100%	2	100%	1	100%

All GPC showed 100% sensitivity to Rifampin. All GPC except one strain of Staphylococcus aureus were sensitive to Vancomycin.(97.4%) Staphylococcus schleiferi and Enterococcus fecalis were totally resistant to fluoroquinolones. Staphylococcus saprophyticus , Staphylococcus warneri and Enterococcus fecalis exhibit multidrug resistance. They were all resistant to cotrimoxazole,cephalexin and penicillin.When compared to coagulase negative Staphylococci,Staphylococcus aureus was more sensitive to chloramphenicol,erythromycin,cephalexin,cotrimoxazole. But it showed less sensitivity than CONS to fluoroquinolones and aminoglycosides.

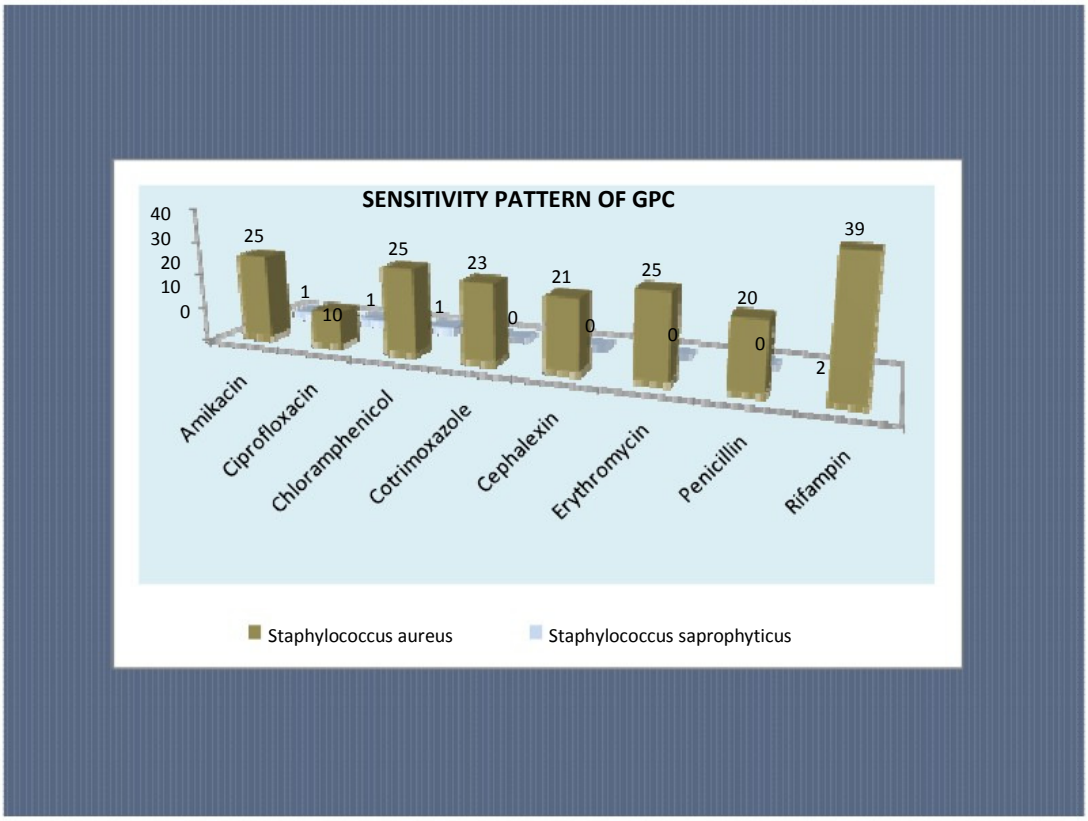

SENSITIVITY PATTERN OF GPC
40
30
20
10
0
25
1
10
1
25
1
23
0
21
0
25
0
20
0
39
2
Amikacin
Ciprofloxacin
Chloramphenicol
Cotrimoxazole
Cephalexin
Erythromycin
Penicillin
Rifampin
Staphylococcus aureus
Staphylococcus saprophyticus

TABLE-14: ANTIMICROBIAL SENSITIVITY PATTERNS OF GRAM NEGATIVE BACILLI (GNB)

Antibiotics	Pseudomonas aeruginosa n =17		Klebsiella pneumoniae n=9		Proteus mirabilis n=10		Escherichia coli n=6		Acinetobacter baumanii n =4	
	Isolate	Percentage	Isolate	Percentage	Isolate	Percentage	Isolate	Percentage	Isolate	Percentage
Amikacin	9	52.9%	8	88.8%	6	60%	4	66.6%	2	50%
Gentamicin	3	17.6%	5	55.5%	2	20%	1	16.6%	2	50%
Cefotaxime	6	35.2%	2	22.2%	4	40%	2	33.3%	1	25%
Ceftazidime	6	35.2%	2	22.2%	4	40%	2	33.3%	1	25%
Ciprofloxacin	3	17.6%	1	11.1%	1	10%	1	16.6%	1	25%
Ofloxacin	5	29.4%	5	55.5%	2	20%	1	16.6%	4	100%
Imipenem	17	100%	9	100%	10	100%	6	100%	4	100%
Cefoperazone/ Sulbactum	13	76.4%	8	88.8%	9	90%	4	66.6%	4	100%

Most of the gram negative bacilli showed high level resistance to third generation cephalosporins. All GNB showed 100% sensitivity to imipenem. Except Pseudomonas aeruginosa and Escherichia coli all GNB showed good sensitivity to cefoperazone sulbactum.

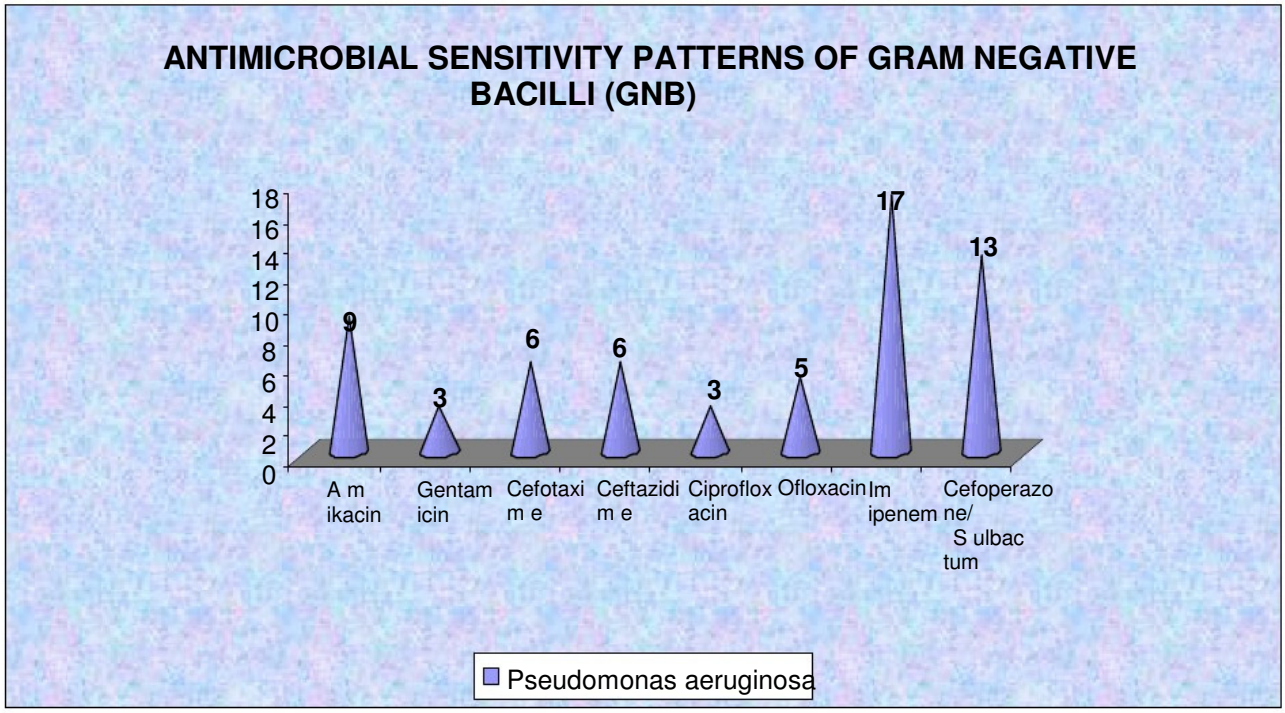

ANTIMICROBIAL SENSITIVITY PATTERNS OF GRAM NEGATIVE BACILLI (GNB)
18
16
14
12
10
8
6
4
2
0
9
3
6
6
3
5
17
13
A m ikacin
Gentam icin
Cefotaxi m e
Ceftazidi m e
Ciproflox acin
Ofloxacin
Im ipenem
Cefoperazo ne/ S ulbac tum
Pseudomonas aeruginosa

TABLE-15: DRUG RESISTANCE MECHANISM AMONG THE PATHOGENS ISOLATED FROM CHRONIC OSTEOMYELITIS

Total Pathogens (n=106)	Number of Multidrug resistant isolate n =43	Percentage	LCL	UCL
Staphylococcus aureus (n=39)				
Methicillin resistant S.aureus (MRSA)	19	48.7%	32.42	54.21
Vancomycin intermediate S.aureus(VISA)	1	2.5%		
Staphylococcus epidermidis (n=11)				
Methicillin resistant Staphylococcus epidermidis	6	54.5%	23.38	83.25
Proteus mirabilis (n=10)				
Extended spectrum β lactamases	6	60%	26.24	87.8
Escherichia coli (n =6)				
Extended spectrum β lactamases	4	66.6%	12.78	66.36
Klebsiella pneumoniae (n =9)				
Extended spectrum β lactamases	7	77.7%	43.79	96.09

Among the 106 aerobic bacteria isolated,43(40.5%) were multidrug resistant. Coagulase negative Staphylococci (54.5%) exhibit more resistance to Methicillin than S.aureus(48.7%). Among Enterobacteriaceae , Klebsiella showed higher level of ESBL production(77.7%).

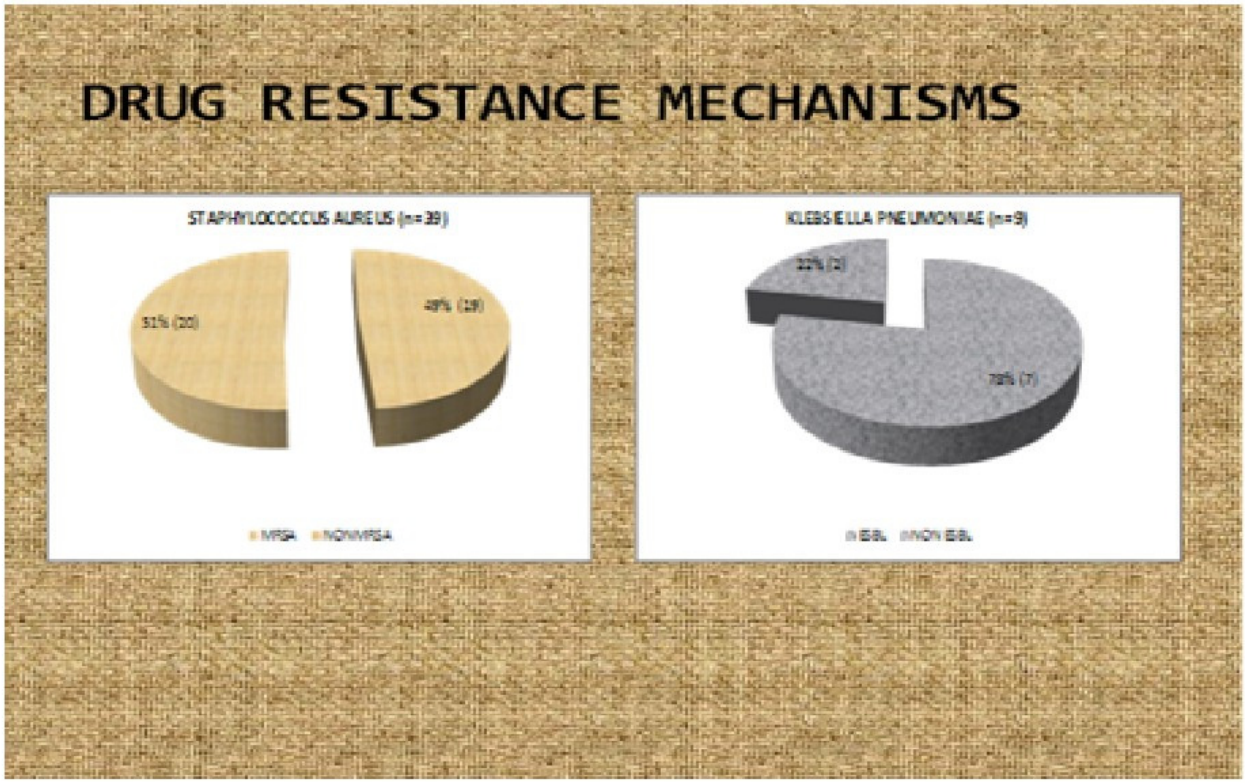

DRUG RESISTANCE MECHANISMS
STAPHYLOCOCCUS AUREUS (n=39)
51% (20)
49% (19)
MRSA
NONMRSA
KLEBSIELLA PNEUMONIAE (n=9)
22% (2)
78% (7)
ESBL
NON ESBL

TABLE-16: DETECTION OF ESBL PRODUCERS AMONG THE GNB

Pathogens	No. of Positive Isolates					
	Screening Test		DDST		PCDDT	
Proteus mirabilis(n=10)	6	60%	6	60%	6	60%
Escherichia coli(n=6)	4	66.6%	4	66.6%	4	66.6%
Klebsiella pneumoniae(n=9)	7	77.7%	7	77.7%	7	77.7%

PCDDT- phenotypic confirmatory disk diffusion test.

DDST- double disk diffusion synergy test.

77.7% of Klebsiella, 66.6% of E.coli, 60% of P.mirabilis, were ESBL producers.

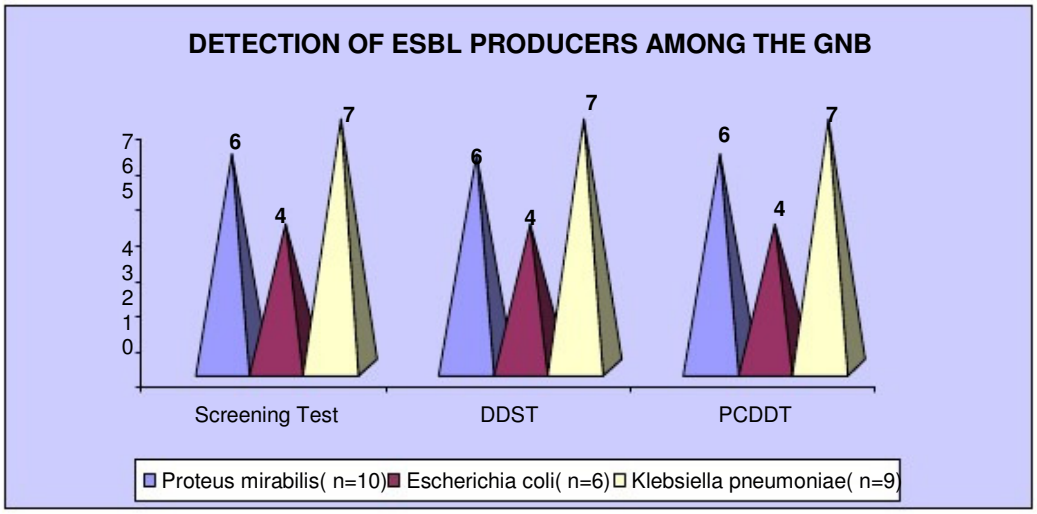


TABLE-17: DRUG SUSCEPTIBILITY PATTERN OF MYCOBACTERIUM TUBERCULOSIS

Drugs	Results
Streptomycin	S
Isoniazid	S
Rifampicin	S
Ethambutol	S
Kanamycin	S
Ethionamide	S
Ofloxacin	S

S- SENSITIVE

Drug susceptibility testing was done in National Institute for research in Tuberculosis –Chetpet. The isolate was sensitive to all first line and second line drugs.

TABLE-18: INTERPRETATION OF MIC OF VANCOMYCIN FOR STAPHYLOCOCCUS AUREUS

Staphylococcus aureus n=39	MIC value	Interpretation
38	≤2 g/l	Sensitive
1	8 g/l	Intermediate

One strain of S.aureus showed intermediate sensitivity to vancomycin

TABLE-19: MIC VALUES OF ANTIFUNGAL AGENTS FOR CANDIDA TROPICALIS [n=1]

Drug	MIC Value(µg/ml)	Interpretation
Fluconazole	2	S
Amphotericin B	0.25	S
Itraconazole	0.25	S
Voriconazole	0.0625	S

S- sensitive

The MIC value of all the four drugs were within their sensitive ranges.

DISCUSSION

This study was conducted in the Institute of Microbiology in association with Institute of Orthopaedics, Rajiv Gandhi Government General Hospital, Chennai- 600 003. 120 patients with chronic osteomyelitis were included for the study.

The age group commonly affected was 31-40 (25%) followed by 21-30 (23.3%) and 12-20(19%)[Table-1].Muggeridge *et al* [73] (2002), Jayasimha *et al* [74] (2003) also showed the similar age groups affected. In all the age groups, males were commonly affected than females due to their occupation. (outdoor work, construction work and high altitude work). In the present study, 97 (80.83%) were males and 23 (19%) were females. Rao and sahu *et al* [75] (1978) also showed nearer percentage of males (77%) in their study. In this study, male to female ratio was (4.2:1)[Table-1]. Samuel L.Turek *et al* [35] also showed similar sex ratio distribution of male to female (4:1).

40% of the patients had duration of the illness from 7 months to 12 months.35.8% of patients had illness ranged from 13-24 months. About 55.8% of patients presented within 1 year. [Table-2]. This was similar to the study conducted by T.P. Srivastava P.C.Sen and V.K.Khanna *et al,* Varanasi, which showed 55%.

Correlation of sex and duration of illness[Table-3] showed p value=0.574. This is not statistically significant.Hence there is no relation between sex and duration of illness.

Correlation of age and duration of illness [Table-4] showed **p value=0.000.The** mean age level in the duration of illness is statistically significant.Group with largest duration of illness had age mean level as 52.3 which is an elevated age mean level.

In this study about 50.8% of patients had compound fracture due to trauma being the commonest predisposing factor [Table-5]. This was slightly higher than the study done by J.Dartnell *et al* [14] who showed 47% of cases were posttraumatic. The next commonest predisposing factor was post surgical (20%) followed by Diabetes mellitus with vascular insufficiency (14.1%), followed by smoking/Alcohol(10.8%) as the commonest predisposing factor. Gustilo *et al* [76] , showed infection rate in the setting of open fracture was 50% which was similar to this study. Sudekamp.N *et al* [77] in his retrospective study showed 56% of cases to be posttraumatic, which was slightly higher than this study. Cherese Thomas *et al* [78] stated that prevalence of Osteomyelitis in diabetic foot ranges from 10-20% .This study showed 14% prevalence.

Tibia (45%) was the most common bone involved, followed by Femur (29.1%) [Table-6].p=0.545.No statistical significance was found between the male and female with respect to different bone sites.Holtom PD *et al* [79] (1999), J.Dart nell *et al* [14] (2012), Muggeridge *et al* [73] (1999) from Australia, V.L.Jayasimha *et al* [74] (2003) from Belgaum also showed similar pattern of involvement.

Cross tabulation of site of bone and age group [Table-7] showed

p=0.140.There is no statistical significance between different age groups and site of bone infection.

Of the samples collected, 63(52.5%) were Sequestrum / Per operative collections of pus and tissue fluids and 57%(47.5%) were swabs[Table-8].

Waldvogel *et al* (1970), Bhattacharya *et al* [80] (1974), Arora *et al* *[81]* (1977) showed culture positivity of 95.3%, 95.2% and 95% respectively. In this study culture positivity was 83.3%[Table-9]. This is in close correlation with Dich *et al* [82] (1975) and Gulati *et al* *[83]* (2008) who showed 85% and 80% respectively. 16.6% yielded sterile cultures. Gulati *et al* (2008) showed in his study that 20% of cultures were sterile. This was slightly higher than this study.

Of the 63 per operative samples/ sequestrum, 59 (93.6%) was monomicrobial and 1 was polymicrobial. In contrast, an increased number of polymicrobial (12.2%) infections were noted in swabs, though monomicrobial infection was the commonest type even in swabs (57.8%)[Table-10].p=0.0000253,statistically **significant.** This strengthens the already known fact that sequestrum and intra operative collections are better samples than swabs, whenever possible.

Out of the total culture positives, 92(76.6%) showed growth in pure form, 8 (6.6%) showed growth in mixed pattern. 6.6% of patients showed mixed growth.[Table-10]. This was closely related to Ako-Nai A.K *et al* *[28]* (2003) study which showed 5% of patients showing mixed infection.

The common organism isolated was *Staphylococcus aureus* (36.7%)[Table-11]. Andres F Zulauaga *et al* [84] 42%(2002), Arora *et al* 42%(1977), Henry *et al [85]* 42.2%(1990), Gulati *et al* [83] 43% (2008) also had lower incidence of *Staphylococcus aureus* isolation. *Pseudomonas aeruginosa* (16%) was the next common organism isolated. This percentage of isolation coincided with Andres F Zuluaga *et al* (2002) who had the same 16% of isolation. Bhattacharya *et al* (1974) and Henry *et al* (1990) had 15% and 17.25% of isolation respectively. The third common organism isolated was *Staphylococcus epidermidis* (10.3%) .Andres F Zuluaga *et al* [84] (2002) showed 10% isolation of *coagulase negative staphylococci.* Ako-Nai *et al [28]* (2003) showed 12.8% isolation rate in his study. Other coagulase negative staphylococci isolated were *staphylococcus schleiferi* (2.8%), *Staphylococcus saprophyticus, Staphylococcus lugdunensis and staphylococcus warneri* (each 1.8%). Other organisms isolated were *proteus mirabilis (9.4%) Klebsiella pneumoniae (8.4%), Escherichia coli (5.6%) Acinetobacter baumanii (3.7%) and Enterococcus fecalis* (1%) Bhattacharya *et al [81]* and Perry *et al* [86] showed 8.7% and 8.5% isolation of *proteus* which was slightly lower than this study. The isolation rate of *Klebsiella* (8.4%) was slightly higher than Henry *et al* who showed 6.9% of isolation. Gulati *et al* and Ako- Nai A.K *et al* showed 5% and 5.1% of *Escherichia coli* in their studies. This isolation rate coincided with this study. Andres F. Zuluga *et al* showed 10% isolation of *Acinetobacter.* This was much higher than this study (3.7%). The isolation of *Enterococcus fecalis* (1%) coincided with the study of Gulati *et al* who also had similar isolation rate of 2%

Out of 92 pure cultures yielded, one showed the growth of acid fast bacilli *Mycobacterium tuberculosis (0.8%)[Table-11].* H.L. Rieder *et al* [87] , has stated that only 1-2% of all tuberculosis cases affect the bone which coincided with this study.

Candida tropicalis(0.8%) [Table-11] was the single fungal agent isolated in this study. This patient had an ulcer in the mouth eroding into maxillary bone. Maria N.Gamaletson *et al [52]* showed that Nonalbicans Candida species caused 35% of Candida Osteomyelitis.

In mixed infections, *Staphylococcus aureus and Pseudomonas aeruginosa* were commonly isolated in combinations. *Escherichia coli and Proteus mirabilis* were also isolated in equal proportions [Table-12].

Staphylococcus aureus which was the commonest bacteria isolated in this study showed 100% sensitivity to Rifampin, 97.4% sensitivity to Vancomycin,64% sensitivity to Amikacin, Chloramphenicol and Erythromycin, 51.2% to Penicillin,51.2% were sensitive to Cefoxitin[Table-13]. Veena Ravi Prakash *et al* [88] (2004) showed 53.3% of MSSA which was slightly higher than the present study (51.2%). Mujumder *et al* [89] (2001) showed 47.10% of MSSA in his study. The second commonest isolate among GPC, *Staphylococcus epidermidis,* showed 100% sensitivity to Vancomycin and Rifampin, 72.7% to Amikacin 54.5% to Chloramphenicol, 45.4% to Penicillin and 27.2% to Quinolones ,Cotrimoxazole.

48.7% of *Staphylococcus aureus* and 54.5% of *Staphylococcus epidermidis* were found to be methicillin resistant[Table-15]. Veena Ravi

Prakash *et al* showed 46.67% of MRSA. Among coagulase negative Staphylococci, *Staphylococcus schleiferi* showed 100% resistance to Ciprofloxacin and Penicillin. Similarly *Staphylococcus saprophyticus and Staphylococcus warneri* were also totally resistant to Cephalexin, Erythromycin and Penicillin. Other than *Staphylococcus epidermidis,* other species of coagulase negative Staphylococci showed multi drug resistance pattern. But all of them showed 100% sensitivity to Vancomycin. Sudha rani J.N.etal [90] (2004) from tirupathi also showed 100% sensitivity of CONS to Vancomycin.

The isolate of *Enterococcus fecalis* showed 100% sensitivity to Rifampin and Vancomycin. It was totally resistant to Ciprofloxacin, Cotrimoxazole, Cephalexin and Penicillin.

Vancomycin sensitivity was detected by Macrobroth dilution method. 38 out of 39 of *S.aureus* showed MIC within sensitive range (<2□g/ml). One isolate showed MIC range of 8 □g/ml, and it was identified as Vancomycin intermediate *S.aureus* (VISA).[Table-18]

Among gram negative isolates, *Pseudomonas aeruginosa* was the most common isolate which showed 100% sensitivity to Imipenem, 76.4% to cefoperazone sulbactum, 52.9% to Amikacin, 35.2% to Cefotaxime and ceftazidime.[Table-14]. Gulati *etal*[83] from Amritsar,showed 89.5% sensitivity to Cefoperazone sulbactum and Amikacin which were higher than this study. *Proteus mirabilis* showed 100% sensitivity to Imipenem, 90% to Cefoperazone/sulbactum, 60% to Amikacin, 30% to Cefotaxime and Ceftazidime.

Gulati *etal* (2004) from Amritsar,showed 89.5% sensitivity to cefoperazone sulbactum and 60.3% to amikacin which were closely similar to the present study. *Klebsiella pneumoniae* showed 100% sensitivity to Imipenem, 90% to cefoperazone/sulbactum and Amikacin[Table-14].Gulati *etal* (2004) showed 89.5% sensitivity to cefoperazone sulbactum which is coinciding with this study.

Among gram negative bacilli, production of extended spectrum lactamases was the mechanism of resistance in them. None of the isolates were found to be MBL producers and AmpC producers. Strains of *Klebsiella,Proteus,E.coli* were screened for ESBL production and confirmed by PCDDT and DDST. [Table-16]

The acid fast bacilli *Mycobacterium tuberculosis* was sensitive to Isoniazid, Rifampicin, Streptomycin, Ethambutol, Kanamycin, Ethionamide and Ofloxacin. [Table-17]

MIC of Fluconazole, Amphotericin B, Itraconazole, Voriconazole for *Candida tropicalis* was determined by Microbroth dilution method .MIC of four drugs were within their sensitivity ranges for the isolate.[Table-19]

SUMMARY

120 patients with chronic Osteomyelitis were taken up for the study. The study showed

- The common age group affected was 20-40 years(48.3%) in both males and females.
- 40% of the patients had duration of illness between 7-12 months, followed by 13-24 months(35.8%).
- The commonest predisposing factor leading to chronic Osteomyelitis was trauma (50.8%) followed by Post surgical infection(20.1%).
- 100 samples showed culture positivit y(83.3%).
- 20 samples showed no growth(16.6%).
- Monomicrobial growth occurred in 92 patients and Polymicrobial growth occurred in 8 patients.
- Polymicrobial growth occurred more in swabs than in Sequestrum or intra operative collections .
- *Staphylococcus aureus* was the most common organism isolated among GPC(36.7%). Among GNB *Pseudomonas aeruginosa* (16%) was commonly isolated.
- One acid fast bacilli and one fungal isolate were also isolated in this study.
- Out of 39 isolates of *Staphylococcus aureus,* 19(48.7%) were MRSA. All strains were sensitive to Rifampin (100%) and all except one(2.5%) were sensitive to Vancomycin.
- Out of 11 isolates of *Staphylococcus epidermidis,* 6 were methicillin resistant(54.5%). All strains were sensitive to Rifampin and Vancomycin

- MIC of vancomycin for *S.aureus* was determined by macrobroth dilution method.
- Single strain identified as VISA had MIC value of 8 □g/ml.
- A single isolate of *Enterococcus faecalis* was sensitive to Vancomycin
- Among 25 gram negative bacilli belonging to Enterobacteriaceae, 17(68%) were found to produce Extended spectrum Beta lactamases(ESBL) by screening method.
- All ESBLS were confirmed by Double disk synergy test (DDST), phenotypic confirmatory disc diffusion test (PCDDT) and E-Strip method.
- MIC of cefotaxime for *E.Coli* and *Klebsiella* were determined by E test.
- All isolates had MIC within the sensitive range.
- *Mycobacterium tuberculosis* was sensitive to all first line and second line drugs.
- The fungal isolate,Candida *tropicalis,* was tested for antifungal susceptibility by determining MIC by Microbroth dilution method.
- MIC of Fluconazole, Amphotericin B, Itraconazole and Voriconazole were within their respective sensitive ranges.

CONCLUSION

This study on 120 patients admitted in orthopaedic wards and those attending outpatient department, Rajiv Gandhi Government General Hospital, Chennai with Chronic osteomyelitis was conducted to study the Predisposing factors, Etiological agents, Antimicrobial Susceptibility Pattern and Drug resistance pattern among the isolates.

Trauma especially road traffic accident with open fracture has been found to be the major predisposing factor. Open fractures leading to Osteomyelitis depends on the type of fracture, the level of contamination, the degree of soft tissue injury and whether local and systemic antimicrobial therapies have been administered. Additional predisposing factors include Post surgical conditions, Diabetesmellitus, Smoking and Alcoholism. The above predisposing factors leads to chronicity of infection, delayed healing and exposure to prolonged antibiotic therapy, resulting in the overgrowth of resistant strains.

56.6% were Aerobic gram positive cocci and 43.3% were Aerobic gram negative bacilli. Among Gram positive cocci, *Staphylococcus aureus* (36.7%)was the commonest pathogen isolated followed closely by *Staphylococcus epidermidis* (10.5%).All gram positive cocci except one were sensitive to Vancomycin and Rifampin. Among gram negative bacilli, all were sensitive to Imipenem and 90% to Cefoperazone sulbactum. Pseudomonas had lower sensitivity (76.4%) to Cefoperazone.

Multidrug resistance was seen in 40.5% of isolates from chronic

Osteomyelitis cases. This factor stresses the need for culture and sensitivit y and choosing appropriate narrow spectrum antibiotic for prolonged therapy (6 weeks). Also, the choice of empirical antibiotics, should be based both on prevalence of local pathogen and antimicrobial susceptibility and on the identification of patient with high risk of developing infections caused by multidrug resistant organism.

As there are various factors in open fracture leading to chronic Osteomyelitis ,each patient has to be routinely monitored after trauma and treatment for developing Osteomyelitis. Treatment given in the early stage will prevent dreadful complications and sequelae.

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APPENDIX

A. STAINS AND REAGENTS

I. Gram staining

Methyl violet (2%)	l0g	Methyl violet in l00ml Absolute alcohol in 1litre of distilled water (primary stain)
Grams Iodine	l0g	Iodine in 20g KI (fixative)
Acetone		Decolourising agent
Carbolfuchsin		1% Secondary stain

II.Acid fast stain

Carbolfuchsin	3g	basic fuchsin in 10 ml of 90-95%ethanol with added 90 ml of 5% phenol
Acid-alcohol	3 ml	conc HCL to 97 ml of 90-95% ethanol.
Methylene blue counterstain	0.3g	methylene blue in 100 ml of dis.water

III. Lactophenol cotton blue stain

Lactic acid	20 ml
Phenol	20ml
Cotton blue (dye)	0.5g
Glycerol	40ml
Distilled water	20ml.

IV. 10% KOH

Potassium hydroxide	l0g
Glycerol	l0ml
Distilled water	80ml

B. MEDIA USED

1. Mac Conkey agar

Peptone	20g
Sodium taurocholate	5g
Distilled Water	1 ltr
Agar	20g
2% neutral red in 50% ethanol	3.5ml
10% lactose solution	l00ml

Dissolve peptone and taurocholate in water by heating. Add agar anddissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake welland mix.Heat in free steam (100°C) for 1 hour, then autoclave at 115°C for 15minutes.

2. Blood agar (5% sheep blood agar)

Peptone	l0g
NaCl	5g
Distilled water	1 Ltr
Agar	10g

Dissolve ingredients in distilled water by boiling, and add 5% sheepblood(sterile) at 55°C adjust pH to 7.4.

3. Chocolate agar

Sterile defibrinated blood	10 ml
Nutrient Agar (melted)	100 ml

When the temperature was about 75°C, sterile blood was added withconstant agitation. After addition of blood, kept in water bath and heating wascontinued till the blood changed to chocolate colour. Cooled to about 50° C and
poured about 15ml into petri dishes with sterile precaution.

4. Sabouraud's dextrose agar

Dextrose	40g
Peptone	l0g
Agar	20g
Distilled water	l000ml
pH	5.5

5. Mueller- Hinton Agar

Beef infusion	300ml
Caeseinhydrolysate	17.5g
Starch	1.5g
Agar	10g
Distilled water	1ltr
pH	7.4

Sterilise by autoclaving at 121ºC for 20 mins

6. Robertson's Cooked Meat Broth

Fresh bullock heart	500g
Water	500ml
Sodium hydroxide	1mol/l 1.5ml
Liquid filtered from	
cooked meat	500ml
Peptone	2.5g
NaCl	1.25g

7.Chrome agar media

Glucose	20gm
Agar	15gm
Peptone	10gm
Chromogenic mix	2 gm
Chloramphenicol	0.5gm

Add to distilled water and mix water. Gently heat. Cool to 45$_0$-50 $_0$c.Pour in sterile petri dish

8.RPMI 1640

(Rose parker memorial institute) with glutamine, withoutbicarbonate, withphenolred as pH indicator:

Dissolve powdered media in 900 ml distilled water.Add MOPS buffer to a final concentration of 0.165 mol/l and stir until dissolved.whilestirring,adjust the pH to 7.0 at 25^{0}c using NAOH.Add additional water to bring media to a final volume of 1L.Filter sterilize and store at 4^{0}c until use.

C. MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION

1.Oxidase Reagent

Tetra methyl p-phenylenediaminedihyrochloride- 1% aqueous solution.

2.Catalase

3% hydrogen peroxide

3.Indole test

Kovac's reagent

Amyl or isoamyl alcohol 150ml

Para dimethyl amino benzaldehyde 10g

Concentrated hydrochloric acid 50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare insmall quantities and store in the refrigerator. Shake gently before use.

4.Christensen's Urease test medium

Peptone	1g
Sodium chloride	5g
Dipotassium hydrogen phosphate	2g
Phenol red	6ml
Agar	20g
Distilled water	1 ltr
10% sterile solution of glucose	l0ml
Sterile 20% urea solution	l00ml

Sterilize the glucose and urea solutions by filtration. Prepare the basalmedium without glucose and urea, adjust to pH 6.8-6.9 and sterilize byautoclaving in a flask at 121°C for 30min. Cool to about 50°C, add the glucose& urea, and tube the medium as slopes.

5.Simmon's Citrate Medium

Koser's medium	1 ltr
Agar	20g
Bromothymol blue 0.2%	40ml

Dispense, autoclave at 121°C for 15 min and allow to set as slopes

6.Triple Sugar Iron medium

Beef extract	3g
Yeast extract	3g
Peptone	20g
Glucose	1g
Lactose	l0g
Sucrose	l0g
Ferric citrate	0.3g
Sodium chloride	5g
Sodumthiosulphate	0.3g
Agar	12g
Phenol red 0.2% solution	l2ml
Distilled water	1 ltr

Heat to dissolve the solids, add the indicator solution, mix and tube. Sterilize at121°C for 15 min and cool to form slopes with deep butts.

7.Glucose phosphate broth

Peptone	5g
Dipotassium hydrogen phosphate	5g
Water	1 ltr
Glucose 10% solution	50ml

Dissolve the peptone and phosphate and adjust the pH to 7.6. Filterdispense in 5ml amounts and sterilize at 121°C for 15min. Sterilize the glucosesolution by filtration and add 0.25ml to each tube.

Methyl Red Reagent

Methyl Red	10mg
Ethyl alcohol	30ml
Distilled water	20ml

VogesProskauer Reagent

Reagent A: Alpha naphthol	5g
Ethyl alcohol	100ml
Reagent B: Potassium hydroxide	40g
Distilled water	100ml

8.Peptone water fermentation test medium.

To the basal medium of peptone water, add sterilised sugars of 1%indicator bromothymol blue with Durham's tube.

Basal medium peptone water

Sugar solutions:

Sugar	1ml
Dislilled water	l00ml
pH	7.6.

9.Mannitol motility medium

Agar	5g
Peptone	1g
Potassium nitrate	1g
Mannitol	2g
Phenol red indicator	
Distilled water	l000ml
pH	7.2

10. Phenolphthalein diphosphate agar

- Sterilize a 1% aqueous solution of sodium phenolphthalein diphosphateby filtration and store at 4ºC
- Add 10ml of this solution to 1000ml melted nutrient agar cooled to 50ºCand pour plates
- Grow the staphylococcus overnight at 37ºC on the medium
- Invert the plate and pour a few drops of ammonia solution SG 0.88 into
- the lid
- Read as positive a culture whose colonies turn bright pink within a few
- minutes. The colour soon fades.

11. Potassium nitrate broth

Potassium nitrate (KN03)	0.2gm
Peptone	5.0gm
Distilled water	100ml

The above ingredients were mixed and transferred into tubes in 5 mlamount and autoclaved.

12. Phenyl alanine deaminase test

Yeast Extract	3g
Dl-Phenylalamine	2g
Disodium hydrogen phosphate	lg
Sodium Chloride	5g
Agar	12g
Distilled water	1 lr
pH	7.4

Distributed in tubes and sterilized by autoclaving at 121° C for 1 5minutes, allowed to solidify as long slopes.

13. Sugar fermentation medium

Peptone	15g
Andrade's indicator	10 ml
Sugar to be tested	20g
Water	1litre

Andrade's indicator is prepared from 0.5% aqueous acid fuchsin towhich sufficient 1M sodium hydroxide has been added to turn the colour of thesolution yellow.

Dissolve the peptone and Andrade's indicator in 1litre of water and add20g of the sugar; sugars to be tested generally include glucose, sucrose, lactoseand maltose. Distribute 3ml amounts in standard test tubes containing aninverted Durham tube. Sterilize by steaming at 100 degree C for 30 min on 3consecutive days.

PROFORMA

 Name :

IP no:

 Age:
 Sex:
 Occupation:
 Address:

Ward:

Presenting complaints:

Duration of illness:

Site of bone:

Predisposing factors:
 Trauma
 Diabetes
 Peripheral vascular disease
 Chronic joint disease
 Alcoholism
 Intravenous drug abuse
 Chronic steroid use
 Immunosuppression
 Tuberculosis
 HIV and AIDS

Past history:

- Prior surgery
- Prior antibiotic therapy
- Steroids
- Immunosuppressive therapy
- Smoking
- Alcoholism

PHYSICAL EXAMINATION:

Laboratory evaluation:

- Biochemical parameters :

  Blood sugar

- Other investigations :

  WBC count

  ESR

  CRP

  Plain X-Ray

  MRI in some cases

- Microbiological investigation:

  Direct examination:

    Gram's stain:

    AFB staining:

    KOH mount:

    Culture :

Aerobic bacterial:

Fungal:

Anaerobic culture:

Antimicrobial sensitivity pattern for bacterial and fungal isolates:

ABBREVIATIONS

ATCC	American type culture collections
CLSI	Clinical & Laboratory Standards Institute
CONS	Coagulase Negative Staphylocci
C.tropicalis	Candida tropicalis
DDST	Double disk diffusion synergy test
E.coli	*Escherichia coli*
ESBL	Extended Spectrum Beta Lactamases
GNB	Gram-negative bacilli
GPC	Gram-positive cocci
MBL	Metallo Beta Lactamases
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSSA	Methicillin Sensitive *Staphylococcus aureus*
M.tuberculosis	Mycobacterium tuberculosis
PCDDT	Phenotypic confirmatory disk diffusion test
RPMI 1640	Rose Parker Memorial Institute 1640
S. aureus	*Staphylococcus aureus*
S. epidermidis	*Staphylococcus epidermidis*
S.lugdenensis	*Staphylococcus lugdunensis*
S.saprophyticus	*Staphylococcus saprophyticus*
S.schleiferi	*Staphylococcus schleiferi*
S.warneri	*Staphylococcus warneri*

ss	AGE SEX	group 1	gp-2	gp-3	gp-4		POST TRAUMATIC	POST SURGICAL	DIABETES	SMOKING	ALCOHOLIC	HAEMATOGENOUS	H/O PTB	SITE OF BONE	II	PUS DISCHARGE	SEQUESTRUM	INTRA OP COLLECTIONS	CULTURE
1	23MY									Y				humerus				Y	PSEUDOMONAS AERUGINOSA
2	70MY													METATARSAL				Y	STAPHYLOCOCCUS AUREUS
3	38MY								Y					SACRUM			Y	Y	E.COLI
4	13FY									Y				FEMUR				Y	STAPHYLOCOCCUSEPIDERMIDIS
5	57MY						Y							FEMUR		Y			PROTEUS MIRABILIS/
										Y					FEMUR	Y			E.COLI
																			STAPHYLOCOCCUS AUREUS
																			PSEUDOMONAS AERUGINOSA
6	55	F			Y		Y							TIBIA			Y	Y	STAPHYLOCOCCUSEPIDERMIDIS
7	32	M	Y				Y							TIBIA			Y		STAPHYLOCOCCUSEPIDERMIDIS
8	16	M												TIBIA					NG
9	17	M			Y							Y		METATARSAL				Y	STAPHYLOCOCCUS AUREUS
10	16	F			Y			Y						FEMUR		Y			NG
11	36	M				Y	Y							TIBIA		Y			PROTEUS MIRABILIS/
12	50	M				Y			Y					TIBIA			Y		E.COLI
13	66	M												RIB			Y		KLEBSIELLA PNEUMONIAE
					Y			Y											ACINETOBACTER
					Y		Y									Y			PSEUDOMONAS AERUGINOSA/
																			STAPHYLOCOCCUS AUREUS
																			PROTEUS MIRABILIS
14	20	M	Y				Y							TIBIA				Y	KLEBSIELLA PNEUMONIAE
15	65	M			Y					Y				FEMUR			Y		STAPHYLOCOCCUS AUREUS
16	23	M			Y		Y							FEMUR		Y			STAPHYLOCOCCUSEPIDERMIDIS
																			KLEBSIELLA PNEUMONIAE
																			STAPHYLOCOCCUS AUREUS
17	40	M			Y			Y						TIBIA			Y		STAPHYLOCOCCUS EPIDERMIDIS
18	23	M			Y		Y							TIBIA			Y		KLEBSIELLA PNEUMONIAE/
19	25	M			Y		Y							CALCANEUM					STAPHYLOCOCCUS EPIDERMIDIS
20	28	M												RIB				Y	STAPHYLOCOCCUSWARNERI
21	22	M									Y			TIBIA				Y	STAPHYLOCOCCUS AUREUS
22	23	M				Y	Y							SKULL			Y		KLEBSIELLA PNEUMONIAE
23	16	M		Y				Y						RADIUS			Y		STAPHYLOCOCCUS AUREUS
24	40	M												FEMUR			Y	Y	ACINETOBACTER BAUMANII
					Y		Y											Y	PROTEUS MIRABILIS
						Y	Y									Y			PROTEUS MIRABILIS
																			STAPHYLOCOCCUS AUREUS
																			STAPHYLOCOCCUS LUGDUNENSIS
25	34	F	Y						Y					SKULL				Y	NG
26	20	M			Y		Y							TIBIA				Y	STAPHYLOCOCCUS AUREUS
27	48	M				Y	Y							TIBIA				Y	STAPHYLOCOCCUS AUREUS/
28	55	M												TIBIA				Y	PSEUDOMONASAERUGINOSA
29	13	F	Y								Y			FEMUR					STAPHYLOCOCCUS AUREUS
30	22	M				Y	Y							TIBIA			Y		KLEBSIELLA PNEUMONIAE
31	17	M			Y					Y				TIBIA					STAPHYLOCOCCUS AUREUS
32	27	M												TIBIA				Y	STAPHYLOCOCCUS WARNERI
33	50	F	Y					Y						TIBIA		Y			STAPHYLOCOCCUS AUREUS
34	36	M		Y			Y							FIBULA		Y			STAPHYLOCOCCUS AUREUS
35	13	M	Y					Y						FEMUR					E.COLI
36	42	M		Y			Y							TIBIA				Y	STAPHYLOCOCCUS AUREUS
					Y		Y									Y			ACINETOBACTERBAUMANII
					Y		Y						Y				Y		STAPHYLOCOCCUS AUREUS
																Y			M.TB
																			NG
																			PROTEUS MIRABILIS
37	40	M			Y		Y							TIBIA				Y	NG
38	30	M	Y							Y				SPINE		Y			STAPHYLOCOCCUS AUREUS
39	22	M			Y		Y							HUMERUS		Y			STAPHYLOCOCCUS AUREUS
40	24	M												TIBIA					STAPHLYOCOCCUS SAPROPHYTICUS
41	34	M		Y						Y				FEMUR			Y		STAPHYLOCOCCUS AUREUS
42	42	M		Y			Y							TIBIA			Y		PSEUDOMONAS AERUGINOSA
43	55	M					Y							TIBIA			Y		NG
						Y													PSEUDOMONAS AERUGINOSA
						Y		Y								Y			PSEUDOMONAS AERUGINOSA
																			STAPHLYOCOCCUS AUREUS
																			STAPHYLOCOCCUS AUREUS
44	38	M			Y				Y					FEMUR		Y			
45	65	M	Y					Y						TIBIA		Y			
46	27	F												DORSALSPINE					
47	26	F			Y							Y	Y	FEMUR			Y		
48	15	M			Y							Y	Y	FEMUR					
49	21	M		Y				Y						DORSALSPINE			Y		
50	58	M												TIBIA					
51	32	M			Y							Y	Y	TIBIA		Y			
52	30	M			Y			Y						FEMUR		Y	Y		
53	47	M			Y			Y						TIBIA		Y			
54	38	M	Y											TIBIA		Y			
55	36	M					Y							TIBIA		Y			
56	45	M		Y			Y							FEMUR					
57	18	M			Y		Y							FEMUR					
58	27	M		Y										FEMUR					
59	60	M																	
			Y					Y									Y		
				Y			Y									Y			
				Y			Y									Y			
				Y															
								Y									Y		

ss	AGE	SEX	group 1	gp-2	gp-3	gp-4		POST TRAUMATIC	POST SURGICAL	DIABETES	SMOKING	ALCOHOLIC	HAEMATOGENOUS	H/O PTB	SITE OF BONE	II	PUS DISCHARGE	SEQUESTRUM	INTRA OP COLLECTIONS	CULTURE
60	40MY								Y						FEMUR			Y		STAPHYLOCOCCUS AUREUS
61	17MY														TIBIA					PSEUDOMONAS AERUGINOSA
62	50MY							Y							FEMUR		Y			STAPHYLOCOCCUS AUREUS
63	67MY							Y							TIBIA		Y	Y		STAPHYLOCOCCUS SCHLEIFERI/
										Y								Y		ENTEROCOCCI
																				ACINETOBACTER
																				STAPHYLOCOCCUS AUREUS
64	41	M			Y				Y						FEMUR/TIBIA			Y		STAPHYLOCOCCUS AUREUS
65	25	F		Y				Y							TIBIA		Y			STAPHYLOCOCCUS AUREUS
66	19	M			Y			Y							FEMUR			Y		STAPHYLOCOCCUS AUREUS
67	14	M						Y							FEMUR					NG
68	36	M		Y				Y							FEMUR		Y			PSEUDOMONAS AERUGINOSA
69	47	M	Y												CALCANEUM		Y			NG
70	18	M			Y					Y					TIBIA		Y			NG
71	54	M	Y						Y						CLAVICLE					PSEUDOMONAS AERUGINOSA
72	25	F													FEMUR			Y		STAPHYLOCOCCUS SCHLEIFERI
73	25	M			Y							Y			TIBIA		Y			NG
74	52	F		Y				Y							TIBIA		Y			NG
75	13	M	Y					Y							FEMUR					NG
76	38	M						Y							CALCANEUM		Y			STAPHYLOCOCCUS EPIDERMIDIS
77	39	M		Y											FEMUR					NG
78	39	M		Y									Y		FEMUR					STAPHYLOCOCCUS LUGDUNENSIS
79	45	M			Y					Y					STERNUM					STAPHYLOCOCCUS AUREUS
80	32	F		Y				Y							FEMUR		Y			STAPHYLOCOCCUS AUREUS/
81	31	F		Y				Y							TIBIA				Y	PSEUDOMONAS AERUGINOSA
82	50	M													TIBIA		Y			STAPHYLOCOCCUS EPIDERMIDIS
					Y					Y									Y	NG
				Y				Y											Y	NG
				Y					Y									Y		KLEBSIELLA pneumoniae
				Y							Y						Y			KLEBSIELLA PNEUMONIAE
																				STAPHYLOCOCCUS AUREUS
																				NG
																				NG
83	13	M	Y						Y						TIBIA			Y		E.COLI
84	40	M			Y			Y							FEMUR			Y		NG
85	45	M													STERNUM					PSEUDOMONAS AERUGINOSA
86	55	F		Y					Y						TIBIA		Y			STAPHYLOCOCCUS AUREUS
87	55	M		Y						Y					METATARSAL			Y		STAPHYLOCOCCUS AUREUS
88	50	M		Y						Y					HUMERUS		Y			E.COLI
89	31	F	Y					Y							TIBIA		Y			STAPHYLOCOCCUS EPIDERMIDIS
90	38	M													METATARSAL		Y			PROTEUS MIRABILIS
91	45	F		Y				Y							FRONTAL BONE		Y			STAPHYLOCOCCUS AUREUS
92	13	M			Y					Y					TIBIA					S.EPIDERMIDIS
93	23	M		Y						Y					HUMERUS					STAPHYLOCOCCUS AUREUS
94	29	F	Y						Y						TIBIA					NG
95	70	M							Y						METATARSAL			Y		PSEUDOMONAS AERUGINOSA
96	38	M		Y											SACRUM		Y			PROTEUS MIRABILIS
97	13	F						Y							FEMUR					STAPHYLOCOCCUS AUREUS
98	57	M			Y							Y			FEMUR					S.SAPROPHYTICUS
99	55	F		Y					Y						TIBIA		Y			PSEUDOMONAS AERUGINOSA
100	29	M		Y											TIBIA					PROTEUS MIRABILIS
101	18	M						Y							TIBIA			Y		STAPHYLOCOCCUS AUREUS
102	19	M			Y			Y							TIBIA		Y			PSEUDOMONAS AERUGINOSA
103	31	M			Y			Y							HUMERUS					S.SCHLEIFERI
104	15	F			Y										TIBIA					STAPHYLOCOCCUS AUREUS
105	23	F	Y						Y						FEMUR		Y			KLEBSIELLA PNEUMONIAE
106	25	M													CALCANEUM					PSEUDOMONAS AERUGINOSA
107	32	M		Y				Y							FIBULA			Y		S.EPIDERMIDIS
108	26	M		Y				Y							TIBIA					CANDIDA TROPICALIS
109	40	F	Y						Y						FEMUR		Y			STAPHYLOCOCCUS AUREUS
110	52	M		Y				Y							TIBIA			Y		NG
111	38	M		Y				Y							METATARSAL					PROTEUS MIRABILIS
112	55	M						Y							TIBIA		Y			PSEUDOMONAS AERUGINOSA
113	44	M			Y			Y							FEMUR		Y			
114	48	M		Y											TIBIA		Y			
115	24	F			Y					Y					RADIUS			Y		
116	48	M								Y					MAXILLA			Y		
117	45	M		Y											TIBIA					
118	36	M		Y							Y				TIBIA		Y			
119	24	M		Y						Y								Y		
120	37	M		Y				Y									Y			
				Y				Y										Y		
				Y				Y									Y			
				Y						Y									Y	
								Y										Y		
								Y									Y			
					Y												Y			
				Y						Y								Y		

AK	GM	CE	CIP	OF	CS	PT				CHLORAMPHENICOL	PENI	COTRI	ERYTHRO	CEPHALE	AC	VANCO	TETRA	IMIPE		OXACILLIN	MRSA	MSSA	MSCONS	MRCONS	ESBL	RIFAMPIN
R	R	R	S	S													S								Y	
S															SS										Y	
S	R	R	S	R							S	S	S	S			S			S		Y			Y	
S	I															SS								Y		
I	I	S	R	R							R		S				RS			R	Y					
I	I	S	S	R	S											S	RS									
S	R																									
R	R	S	R	S												SS	RS								Y	
S	R									S	R	S	S	S						R	Y					S
R	R														SSS										Y	
											R	I	R	S						R	Y			Y		S
										S	R		R	R						R	Y			Y		S
S				R							R	R	R	S		S	S			R	Y					S
S	S	R	R	R	S														S						Y	
S	R	R	R	R	S														S						Y	
S	S	R	R	R	S														S						Y	
R	R	R	R	S	S														S						Y	
R																										
S											R	S	R		S	S				R	Y					S
S	R	R	R	S	S														S						Y	
S	R	R	R	R	S														S						Y	
S											R	S	S	R		S				R	Y					S
											R	S	S	S		S				R	Y			Y		S
	S	R	R	S	S														S						Y	
				R							R				R	S	R			R	Y					S
R											R	S			R									Y		S
S	R	S		S	S														S							
R			S								R	R	R	R						R	Y			Y		S
R										S	R	R	R	R	R	S				R	Y			Y		S
S											S	S	S	S	S	S				S		Y				S
R	R	R		S	S												R	S							Y	
R										R									S							
S	R	S		R	S	S													S						Y	
R	R	R		R	S														S							
			R								S	S	S	S	S					S	Y					S
				R						S	R	R	R	R	R	S	R			R	Y					
R											R	S	S	R	R					R	Y					S
R										S	R	R	R	R	R	S				R	Y					S
R	R	S	R	S	S	S													S						Y	
S											S	S	S	S						S						
R	R	R	R	R	S					S	S	S	S	S		S				S						S
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R	R	R	S	R	S						R	S	S	S		S				S						S
S											S	S	S	S						S						
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R	R	R	R	R	S						S	S	S	S		S				S					Y	
S			R								S	S	S	S					S	S		Y				S
S			S								S				R					S		Y				
S			S								S									S		Y				
S			S									S	S	S		S			S							
	S	R		S	S																				Y	
R	R	S	R	R	S														S							
R	R	R	R	R	S														S						Y	
S			S								R	S	S	S						S							
R			R								R	S	R	R		S				S						S

AK	GM	CE	CIP	OF	CS	PT			CHLORAMPHENICOL	PEN	COTRI	ERYTHRO	CEPHALE	AC	VANCO	TETRA	IMIPE		OXACILLIN	MRSA	MSSA	MSCONS	MRCONS	ESBL	RIFAMPIN
R	R									S	S	S	S		S		S		S		Y				S
S	R	R	R													S										
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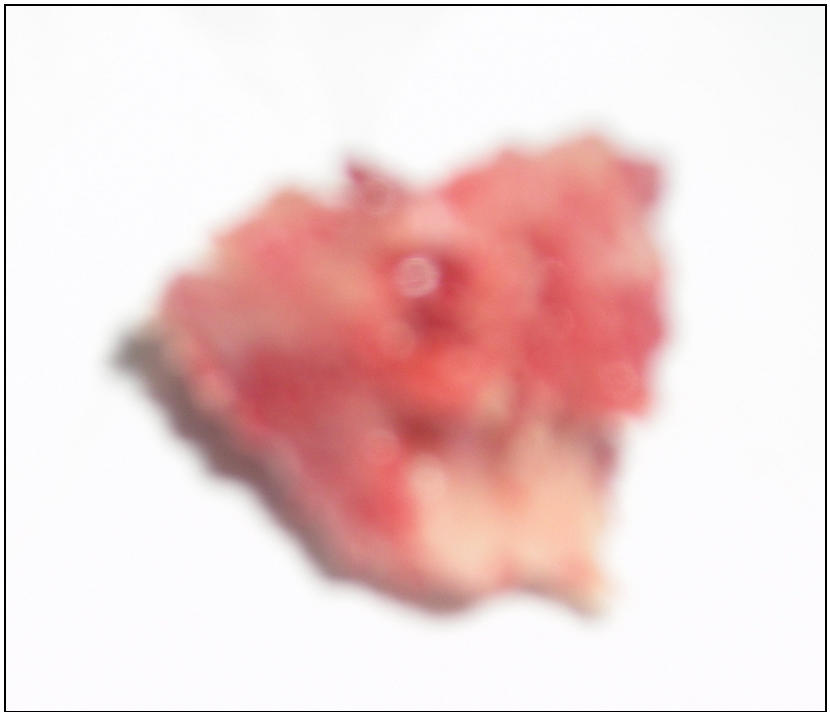
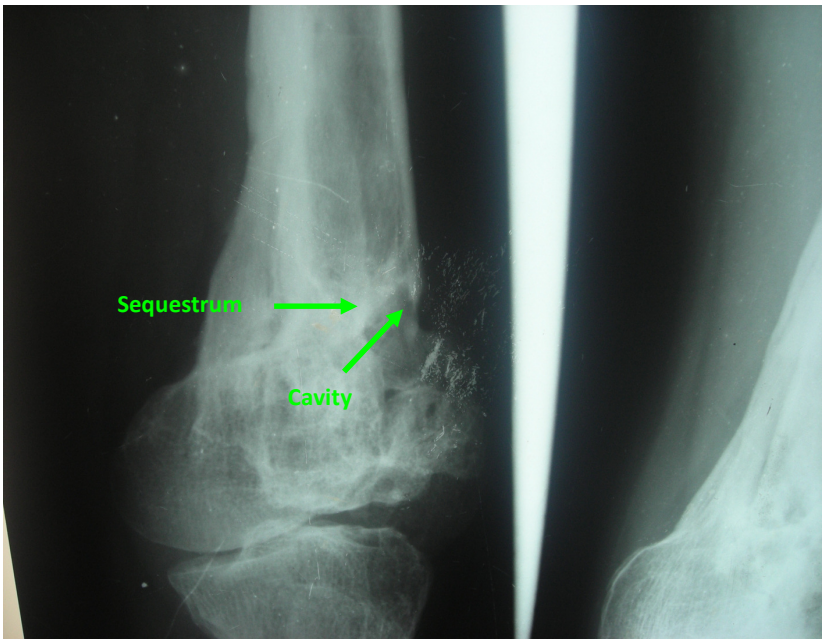
SINUS WITH DISCHARGE

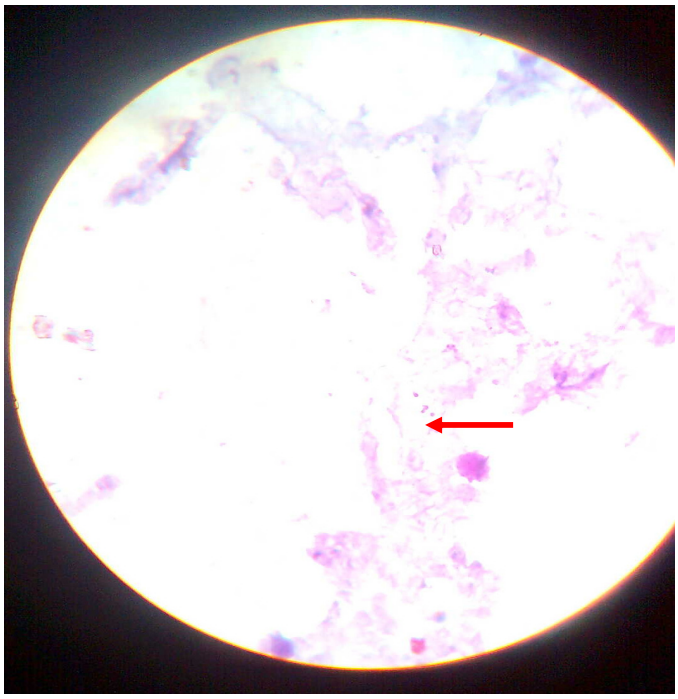
SEQUESTRUM



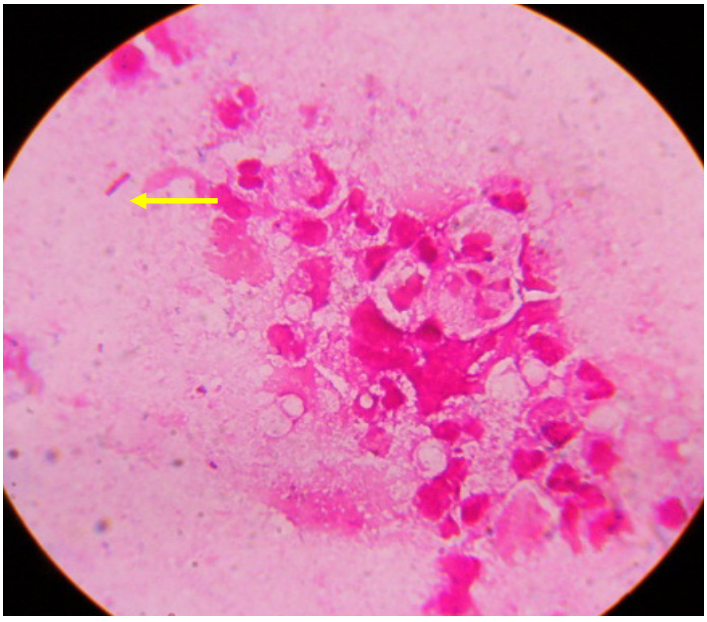
X-RAY SHOWING CAVITY AND SEQUESTRUM



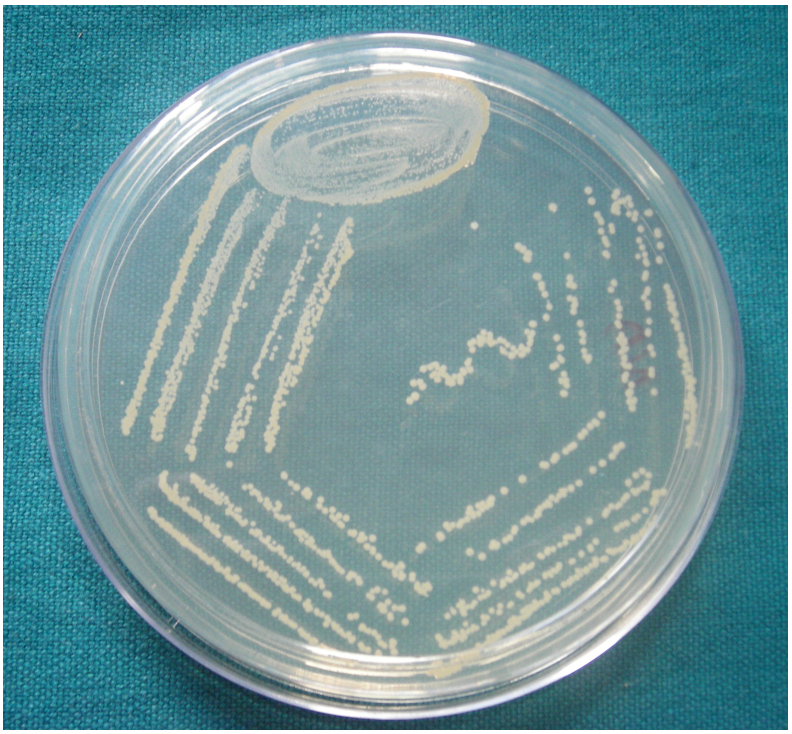
DIRECT GRAM STAIN SHOWING NEUTROPHILS AND GRAM POSITIVE COCCI IN CLUSTERS (STAPHYLOCOCCUS AUREUS)



DIRECT GRAM STAIN SHOWING PLENTY OF NEUTROPHILS WITH GRAM NEGATIVE BACILLI (PROTEUS MIRABILIS)



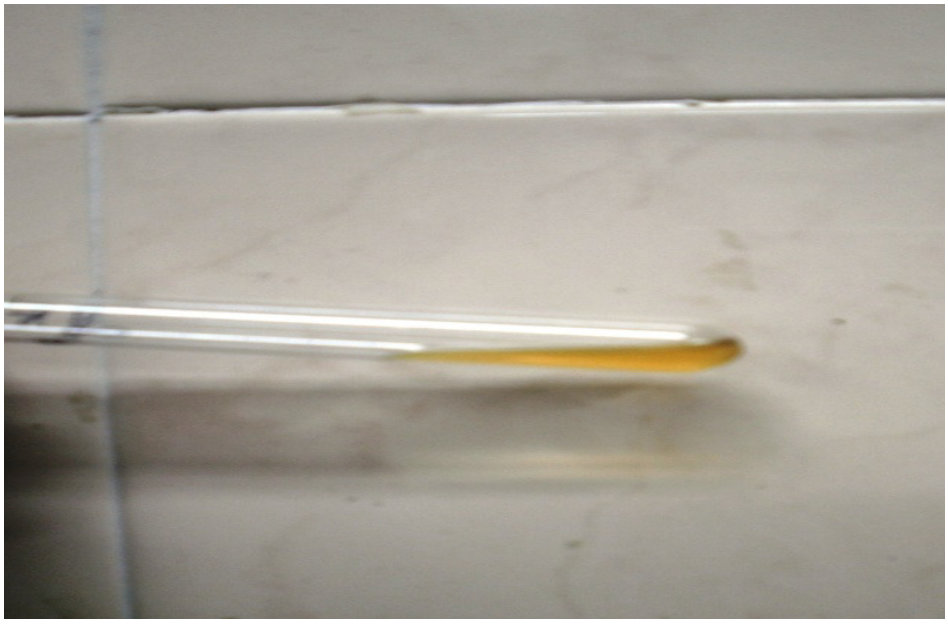
STAPHYLOCOCCUS AUREUS IN NUTRIENT AGAR PLATE WITH GOLDEN YELLOW PIGMENT



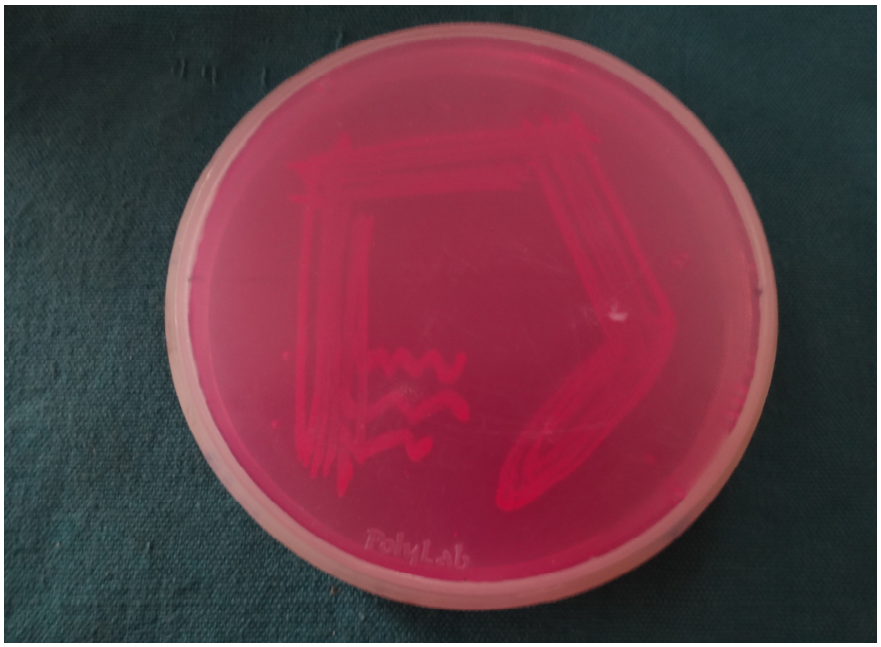
STAPHYLOCOCCUS AUREUS IN BLODO AGAR PLATE WITH β HEMOLYSIS

TUBE COAGULASE-POSITIVE

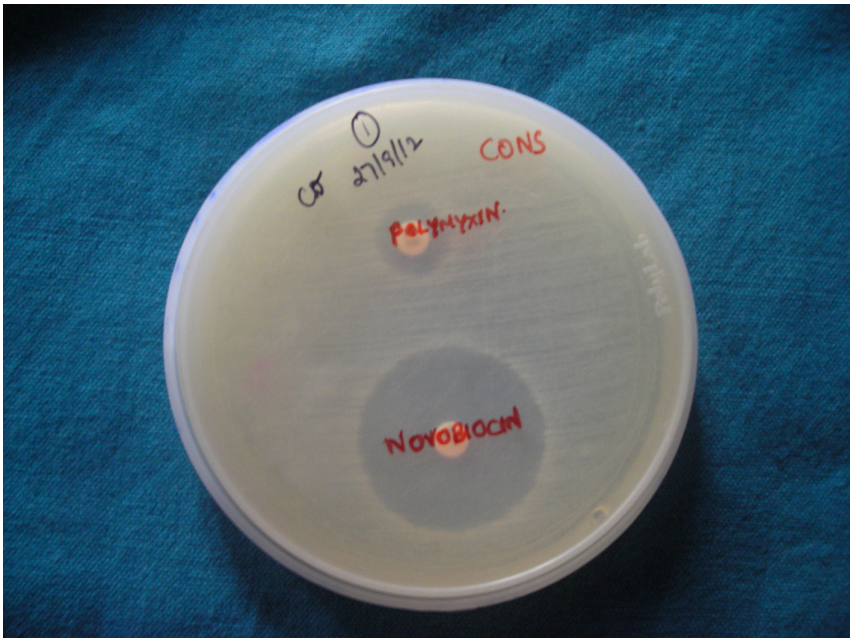
TUBE COAGULASE-NEGATIVE



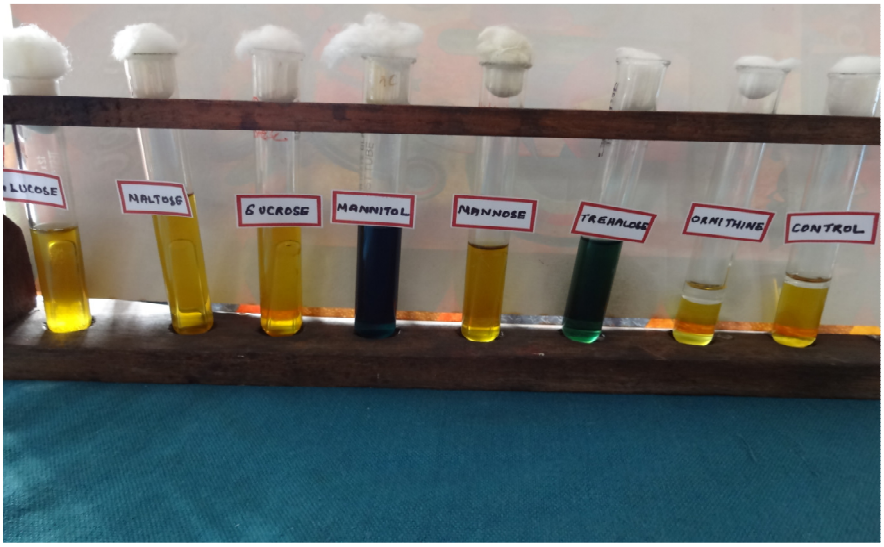
PHOSPHATASE TEST-AFTER EXPOSURE TO AMMONIA VAPOUR



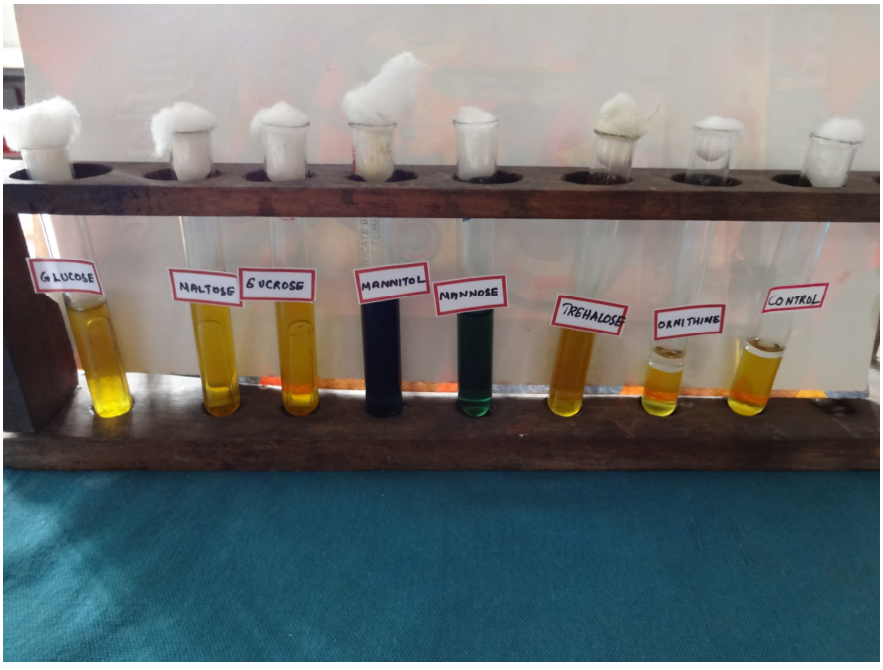
NOVOBIOCIN SENSITIVE AND POLYMYXIN RESISTANT S.EPIDERMIDIS



BIOCHEMICAL REACTIONS OF STAPHYLOCOCCUS EPIDERMIDIS



BIOCHEMICAL REACTIONS OF STAPHYLOCOCCUS WARNERI



BIOCHEMICAL REACTIONS OF STAPHYLOCOCCUS SAPROPHYTICUS

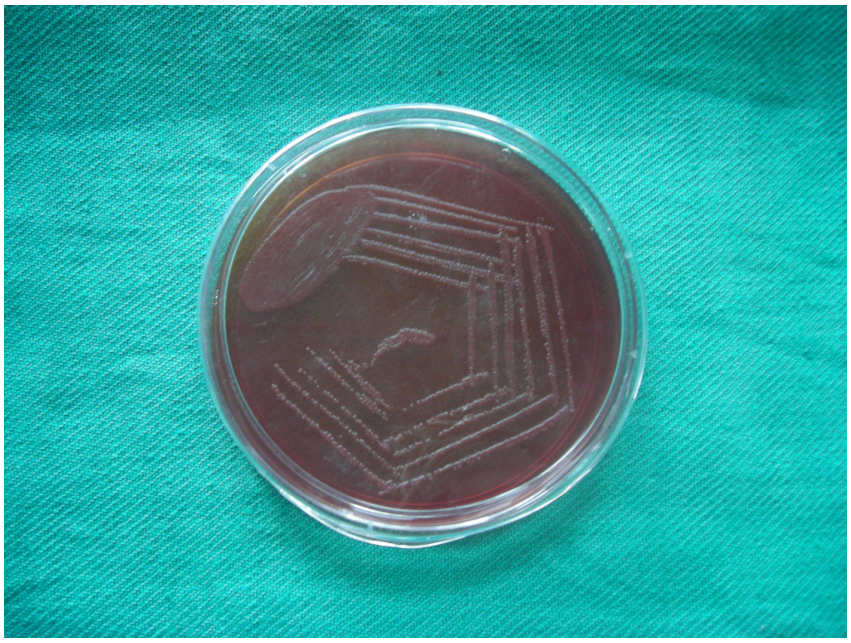
BIOCHEMICAL REACTIONS OF STAPHYLOCOCCUS SCHLEIFERI

BIOCHEMICAL REACTIONS OF STAPHYLOCOCCUS LUGDUNENSIS

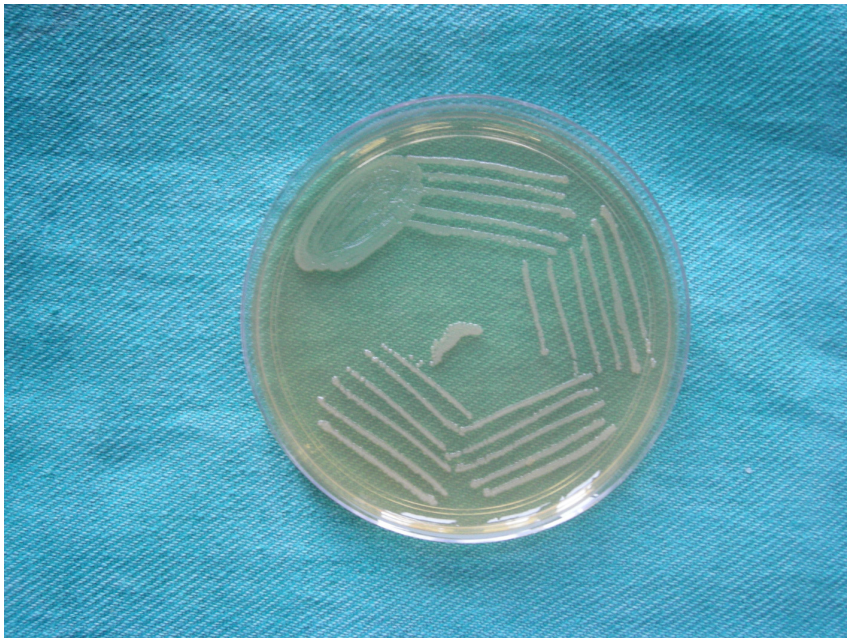
GROWTH OF KLEBSIELLA PNEUMONIAE ON MAC CONKEY AGAR

BIOCHEMICAL REACTIONS OF KLEBSIELLA PNEUMONIAE

GROWTH OF PSEUDOMONAS AERUGINOSA ON MACCONKEY AGAR

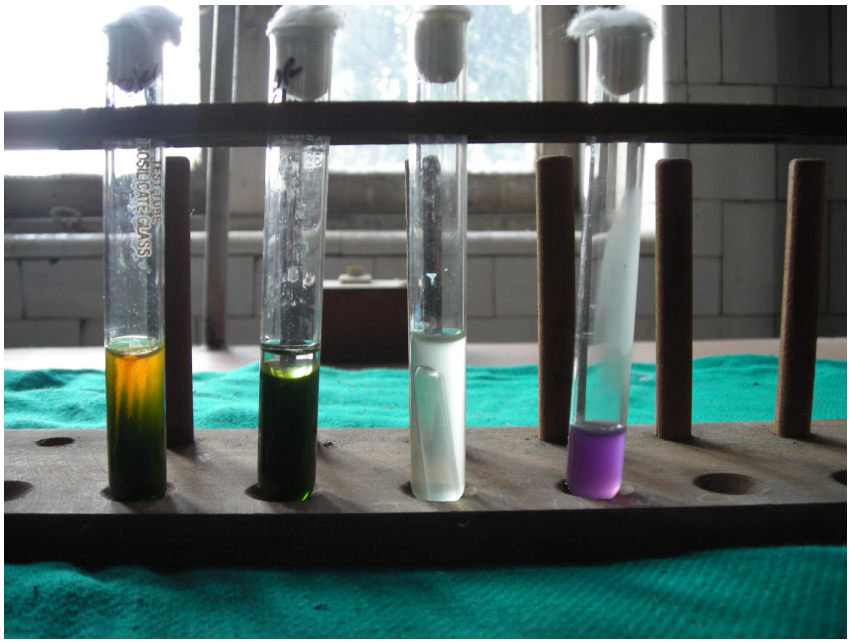


GROWTH OF PSEUDOMONAS AERUGINOSA ON NUTRIENT AGAR

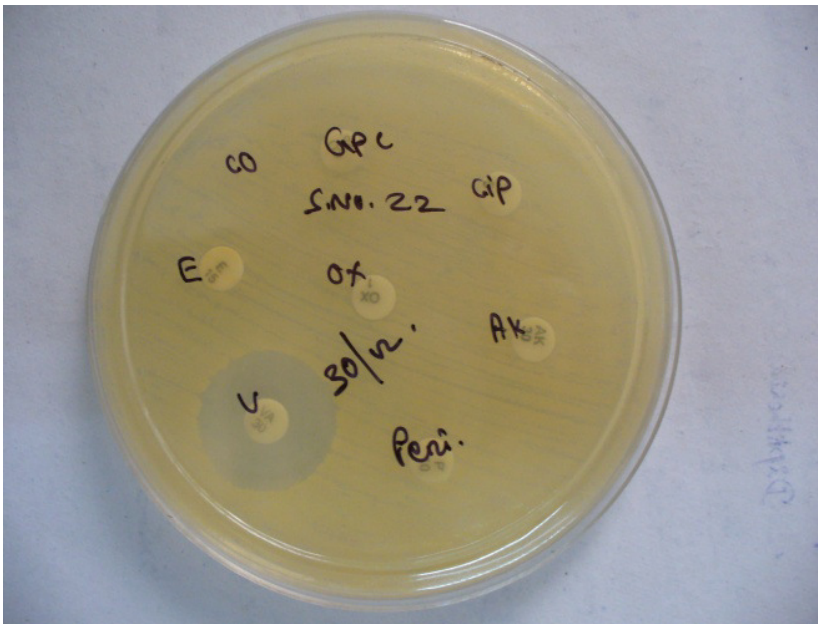


BIOCHEMICAL REACTIONS OF PSEUDOMONAS AERUGINOSA

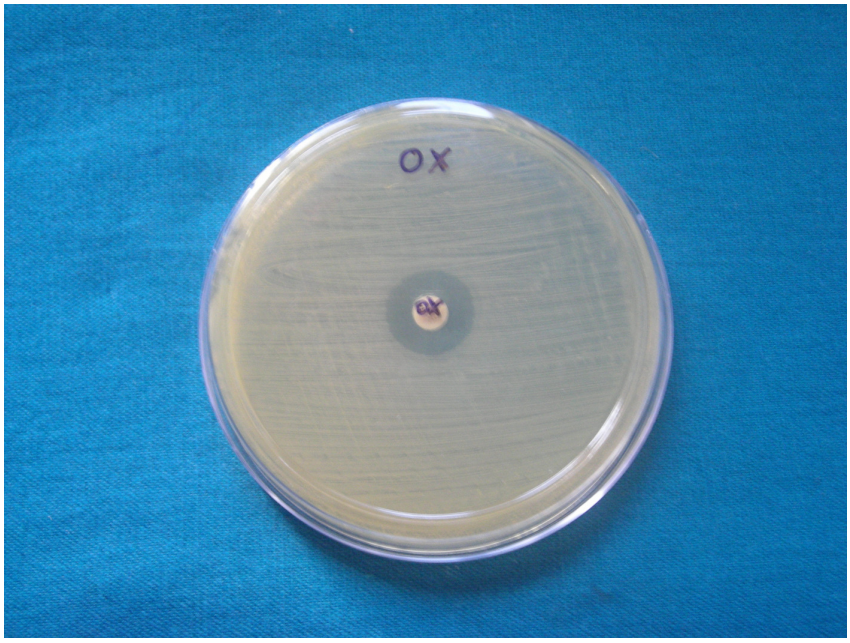
BIOCHEMICAL REACTIONS OF ACINETOBACTER BAUMANII



METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

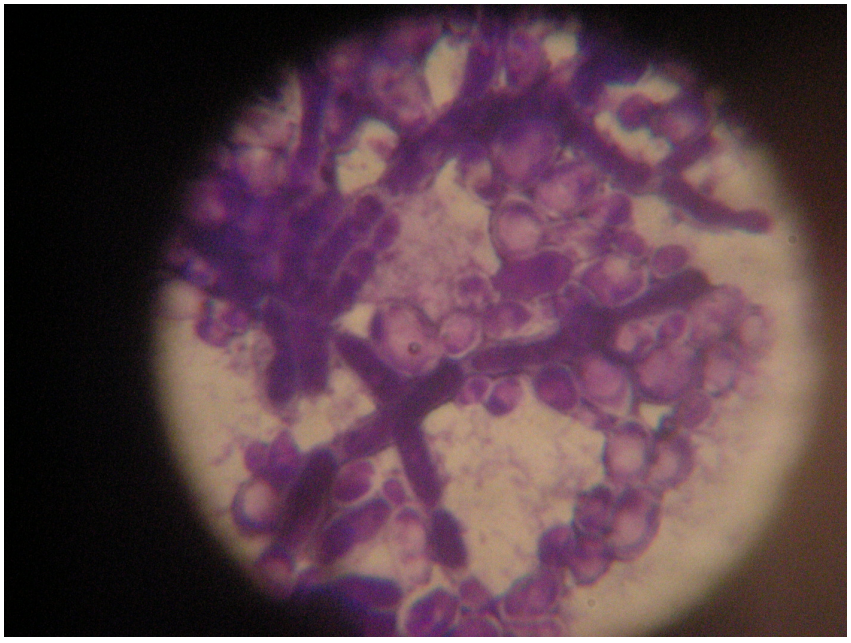


METHICILLIN SENSITIVE STAPHYLOCOCCUS AUREUS

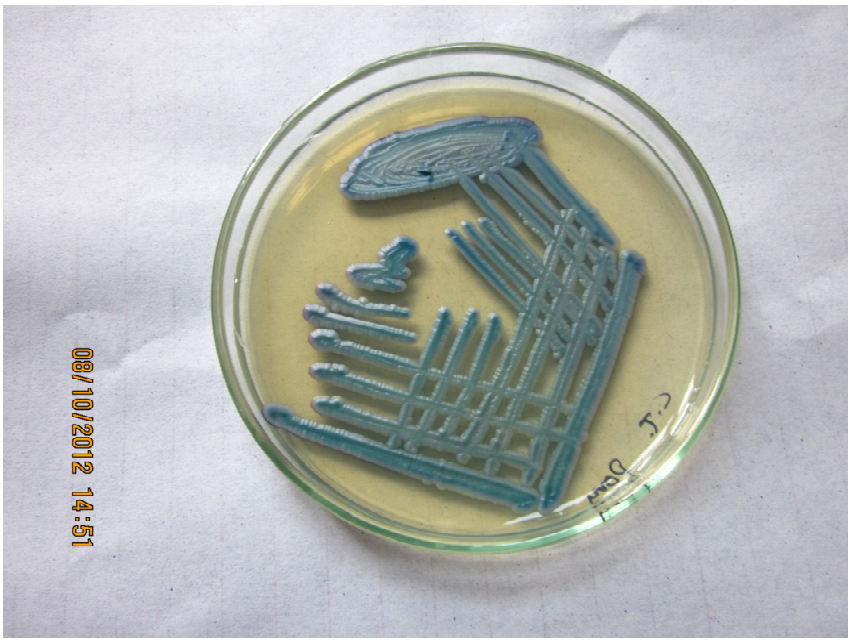


GROWTH OF CANDIDA TROPICALIS ON SABOURAUD'S DEXTROSE AGAR

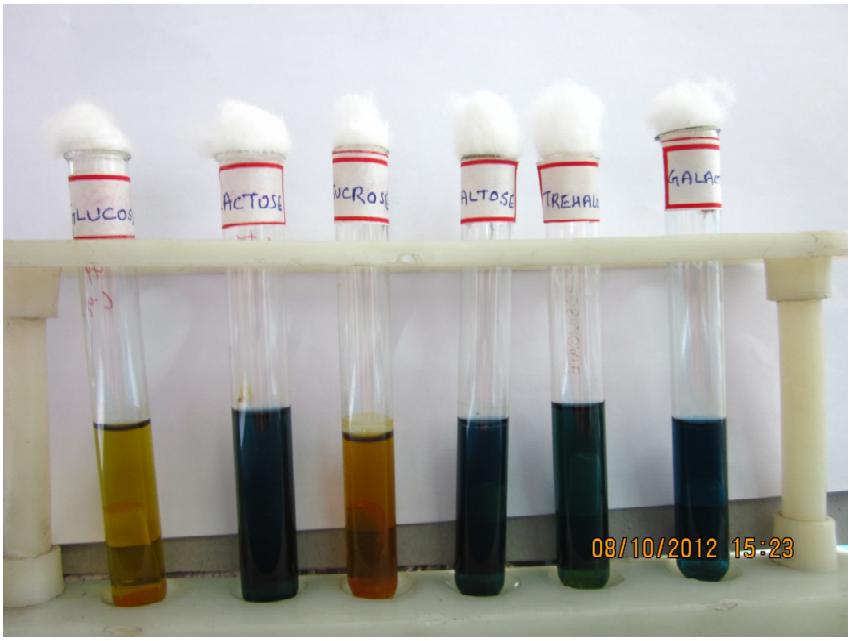
GRAM STAIN OF CANDIDA TROPICALIS SHOWING PSEUDOHYPHAE



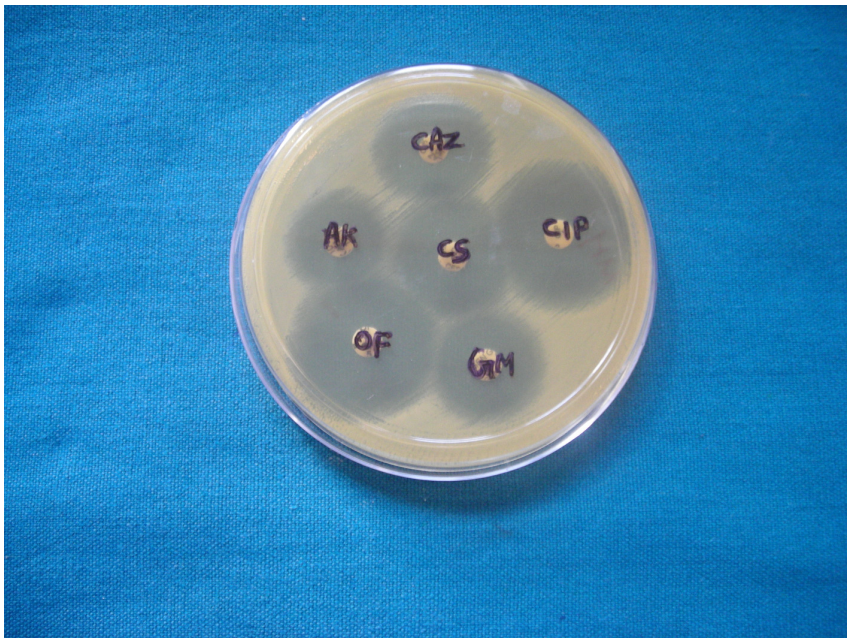
CANDIDA TROPICALIS ON CHROMEAGAR PLATE



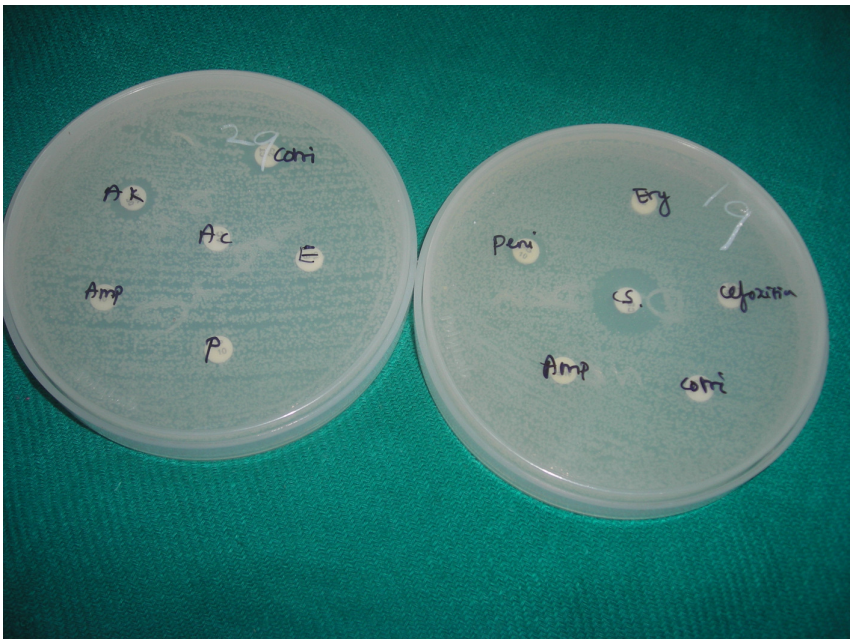
SUGAR FERMENTATION OF CANDIDA TROPICALIS



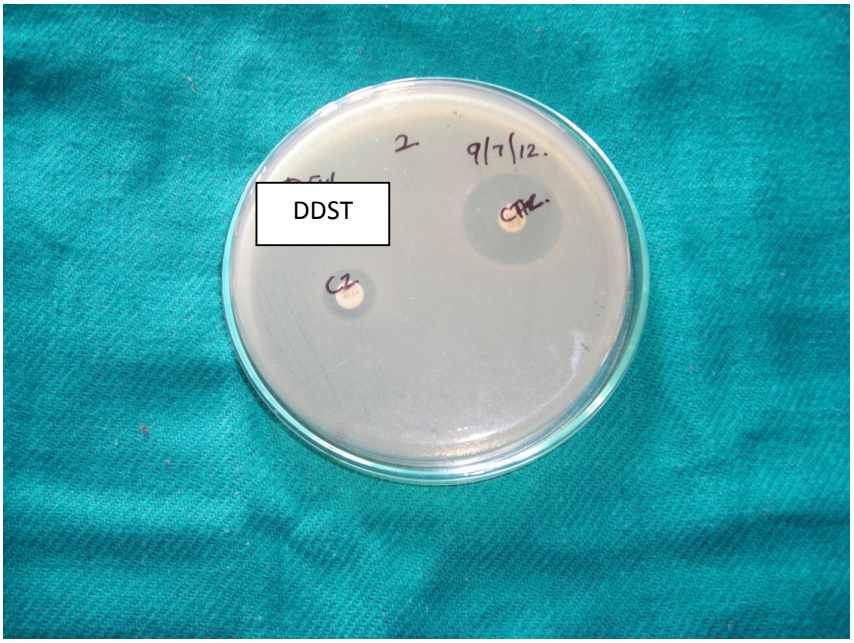
ANTIMICROBIAL SENSITIVITY PATTERN OF ESCHERICHIA COLI



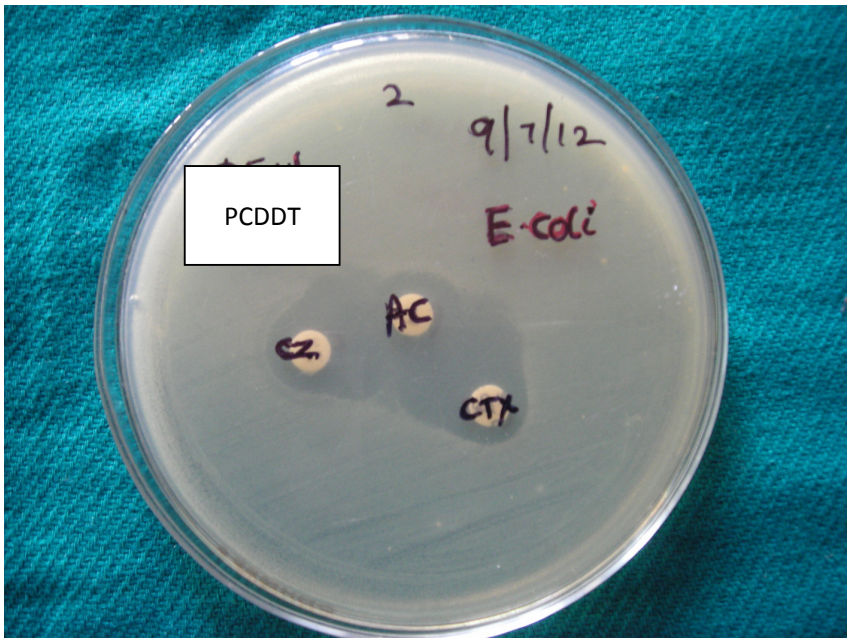
ACINETOBACTER BAUMANII RESISTANT TO PENICILLIN, AMPICILLIN ERYTHROMYCIN



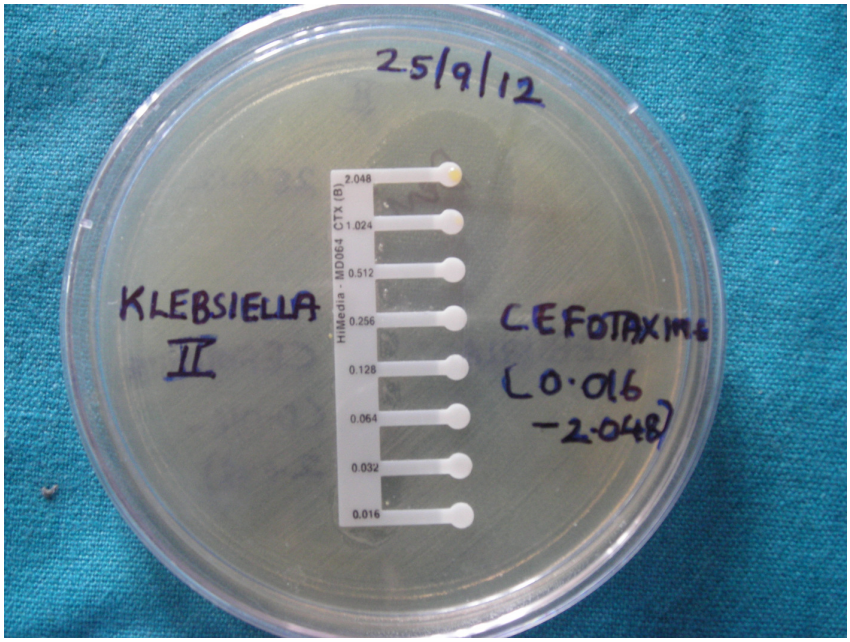
DOUBLE DISK DIFFUSION SYNERGY TEST



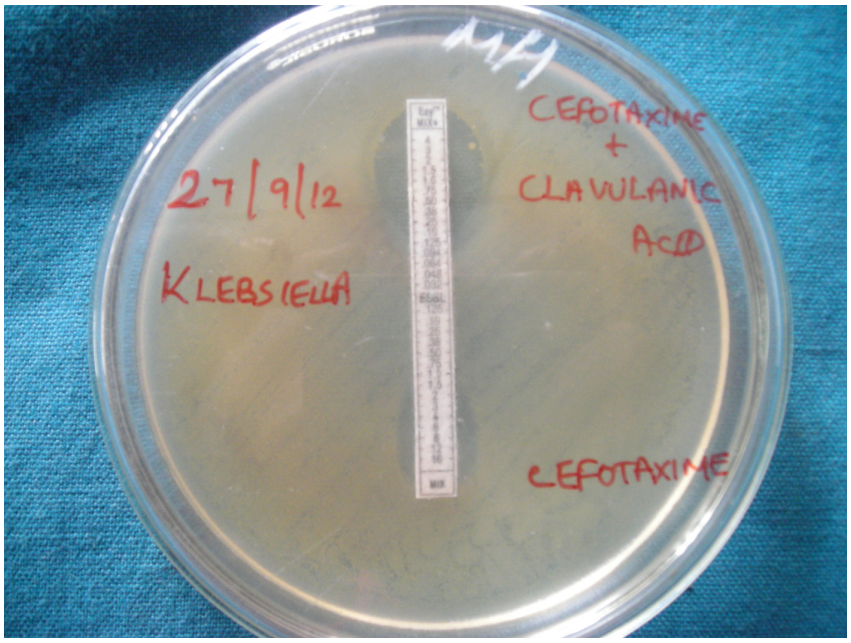
PHENOTYPIC CONFIRMATORY DISK DIFFUSION TEST



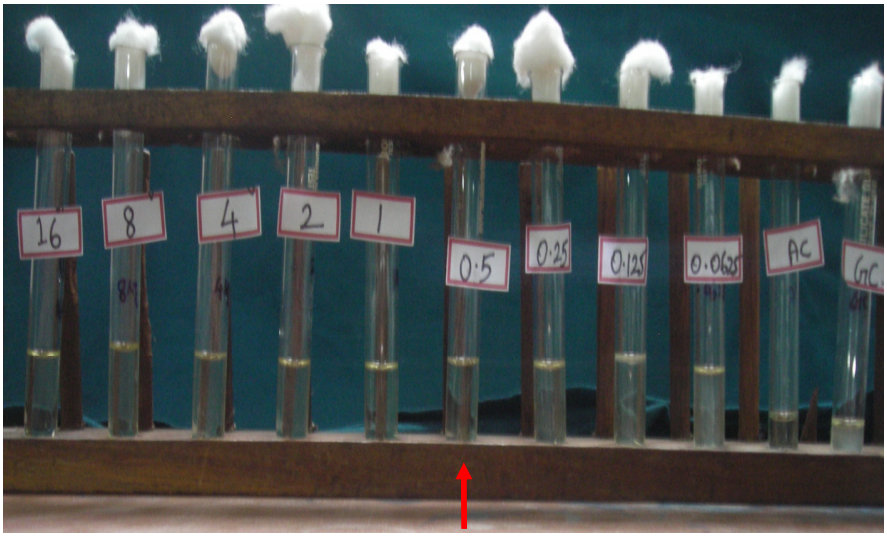
MIC OF CEFOTAXIME FOR KLEBSIELLA HI-COMB METHOD



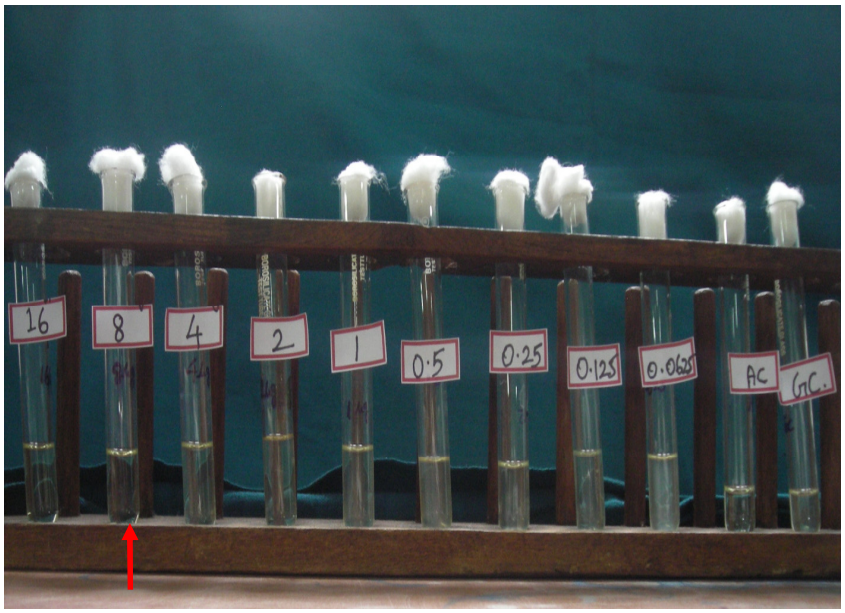
ESBL DETECTION BY E-STRIP METHOD



MINIMUM INHIBITORY CONCENTRATION OF STAPHYLOCOCCUS AUREUS BY MACROBROTH DILUTION METHOD.MIC-0.5µG/ML



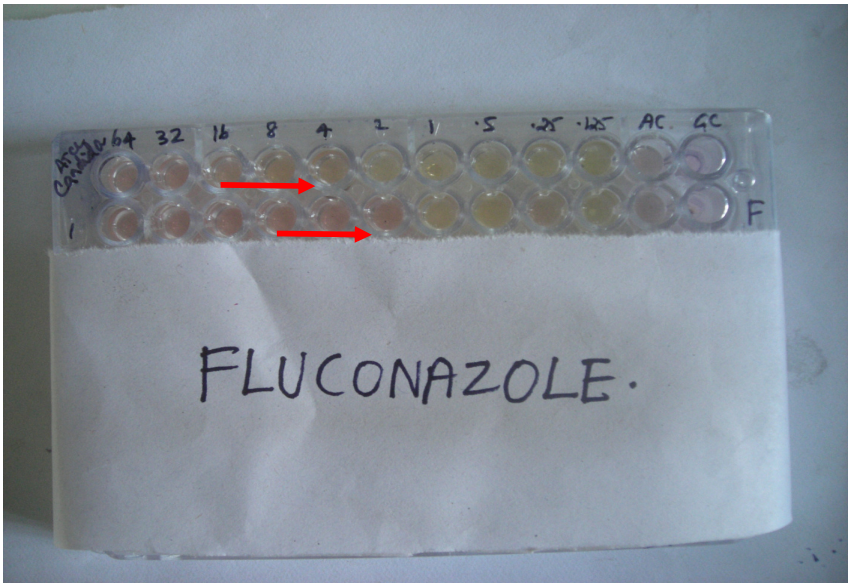
MINIMUM INHIBITORY CONCENTRATION OF VANCOMYCIN FORVANCOMYCIN INTERMEDIATE STAPHYLOCOCCUS AUREUS (VISA) MIC-8µG/ML



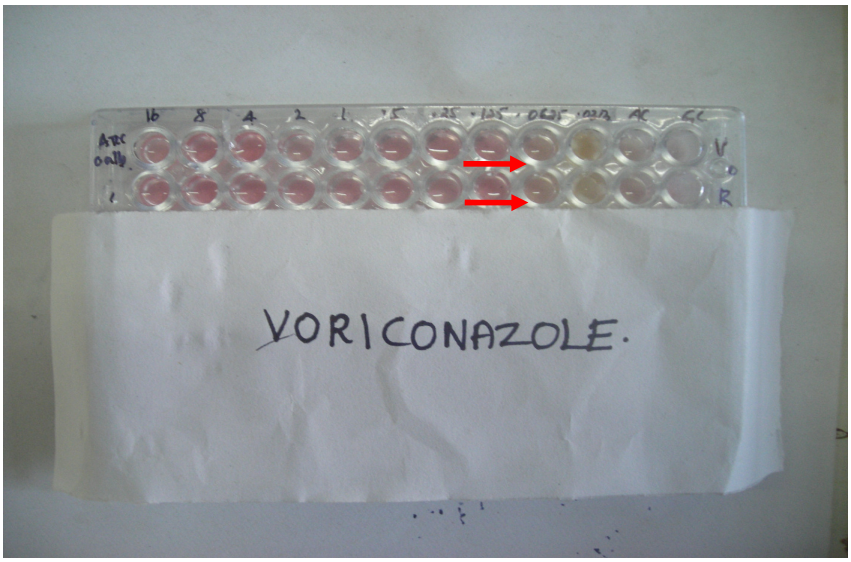
MINIMUM INHIBITORY CONCENTRATION OF ITRACONAZOLE FOR CANDIDA TROPICALIS

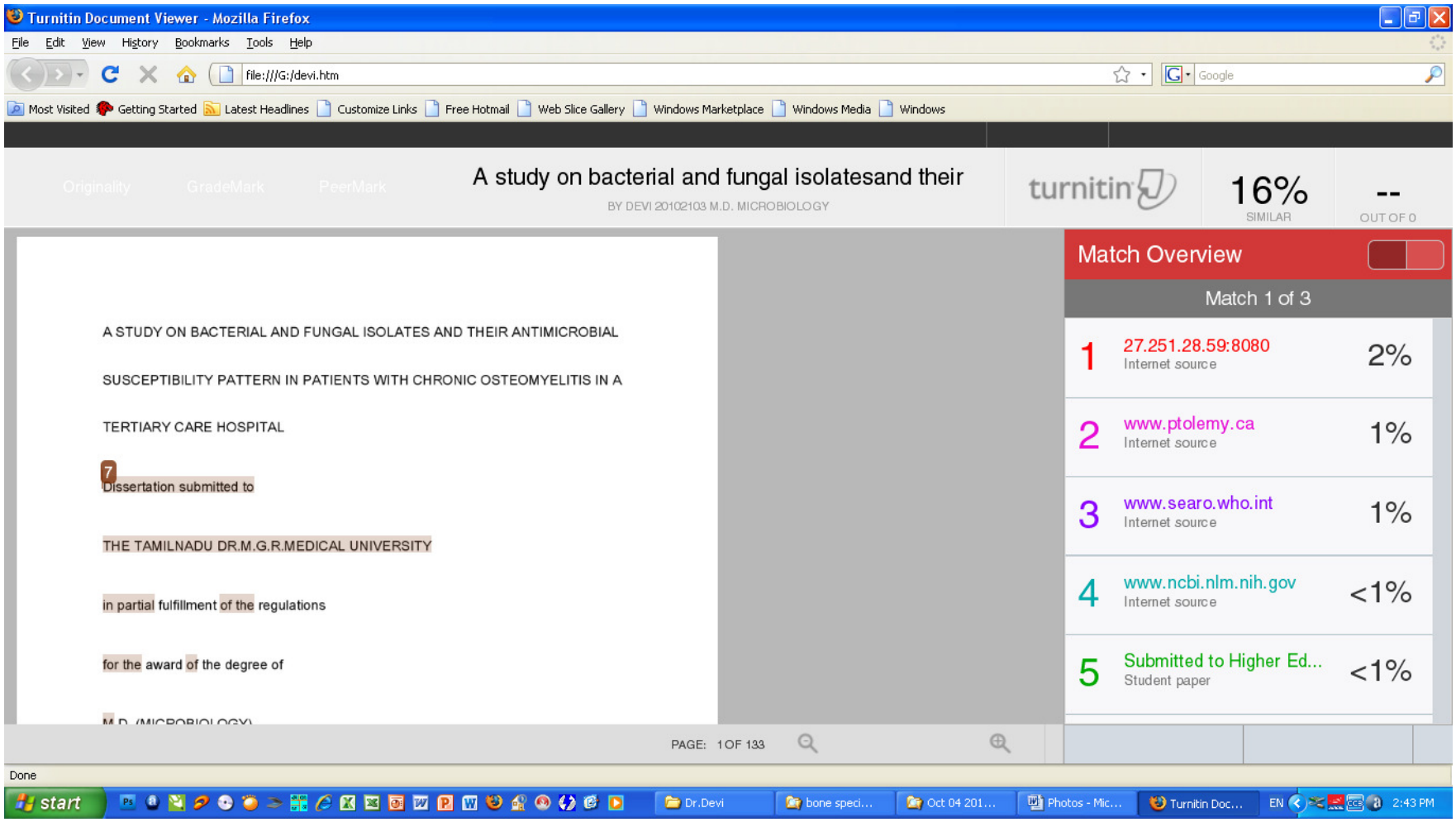
MINIMUM INHIBITORY CONCENTRATION OF AMPHOTERICIN-B FOR CANDIDA TROPICALIS

MINIMUM INHIBITORY CONCENTRATION OF FLUCONAZOLE FOR CANDIDA TROPICALIS



MINIMUM INHIBITORY CONCENTRATION OF VORICONAZOLE FOR CANDIDA TROPICALIS

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